Changes in Cell Migration and the Rate of Cell Loss from Crypts in the Descending Colon of Mice Treated with 1,2-Dimethylhydrazine

by

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ABBREVIATIONS

ANOVA	analysis of variance
body wt.	body weight
cells/hour	cell positions per hour
C	centigrade
Ci	curie (2.22×10^{12} DPM)
DMH	1,2- dimethylhydrazine
EDTA	ethylene diamine tetracetic acid
g	gram
HU	hydroxyurea
i.p.	intraperitoneal
kg	kilogram
LI	labeling index
M	molar
MI	mitotic index
mg	milligram
p	probability
pH	$-\log H^+$ concentration
s.c.	subcutaneous
S.E.M.	standard error of the mean
TdR	thymidine
μm	micrometers (10^{-6} meters)
VB	vinblastine

I. Background

A. Biology of the Descending Colon Crypts

The epithelial lining of the gastrointestinal tract in mammals is constantly renewed and is held in a steady state by a balance between the birth of new cells in the mucosal crypts and the loss of old cells by sloughing from the luminal surface. Bizzozero in 1888 (4) observed mitotic cells in the intestinal crypts and surmised that new cells replace cells damaged by the intestinal contents. Leblond et al. (32) confirmed with radioautography that ^{32}phosphate-labeled cells divide in the base of the crypts and migrate upward replacing cells that are sloughed from the epithelial surface. More recently, studies with tritium-labeled thymidine which is incorporated specifically into synthesizing DNA, have been performed to elucidate the kinetics of cell renewal and migration, to measure the phases of the cell cycle and to determine the maturation of intestinal epithelial cells (8,11,12,16,25,42,46,51). Quastler and Sherman (42) demonstrated that two distinct cell populations could be identified in the small intestinal epithelium. One population of cells was found to undergo a regular proliferative cycle of DNA synthesis and division before the daughter cells migrate onto the villi and differentiate into the second population of mature absorptive cells. In a similar manner, Lipkin and Quastler (34) defined the colonic crypt as the functional unit for the study of intestinal crypt populations. When analyzed histologically, it

consists of a single column of cells extending from the base of the crypt to the crypt-surface junction. The column of cells can be divided into a proliferative zone in the lower portion and a maturation zone above which extends to the crypt surface. The basally located stem cells differentiate into mucuous, columnar, argentaffin or enteroendocrine cells during their lifespan in the descending colon of rodents (11,57).

The number of cells in the crypt column of the mouse descending colon varies among strains and ranges from 26 to 38 cells in length and approximately 22 cells in crypt circumference (8,11,12,16,18,25,44). Crypt cells labeled with ^{3}H-thymidine have been shown to migrate from the proliferative zone to the crypt surface in 3 days and labeled pyknotic cells are extruded from the surface epithelium after 4 days (11).

B. Cell Proliferation

The dividing cells in the crypts of the descending colon are normally restricted to the lower one-half of the crypt, and this region is referred to as the zone of proliferation (16,24,40). The majority of cell division takes place in the upper portion of the proliferative zone (11,34), whereas the cells in the extreme base of the crypt are thought to be a slowly dividing stem cell population that gives rise to daughter cells which divide more rapidly as they migrate to higher cell positions in the crypt (11). The length of the cell cycle of crypt cells in the mouse colon has been estimated to be from 15 to 26 hours (12,16,34,44,53). The cell cycle in these studies was estimated by

measuring changes in the fraction of labeled mitoses after a pulse label of ^{3}H-thymidine. Discrepancies in the total length of the cell cycle exist because the population of dividing cells rapidly becomes asynchronous. One known factor that contributes to this asynchrony is the diurnal fluctuation in proliferative activity of the mitotically-active cells. Cells in the colon have been shown to reach a zenith of proliferative activity at 1200 hours and a nadir at 2400 hours (8,25).

The mechanisms which control cell proliferation in the intestinal crypts are not well understood, but several factors are known to influence the rate of cell proliferation. Increasing age has been associated with a decreasing index of DNA synthesis in colonic epithelium that may be due to a prolongation of the G-1 phase of the cell cycle (53). Nutritional status appears to be a factor, since fasting and refeeding animals result in a marked depression of cell proliferation that is followed by a surge of proliferative activity (24,50). Hormonal status also appears to play an important role in proliferative activity, since elevation of estrogen levels will decrease incorporation of ^{3}H-thymidine into DNA in mouse colonic mucosa (27). Neural regulation of intestinal cell proliferation appears to exert its effect through adrenergic nerve endings because a post-ganglionic sympathectomy results in a decreased rate of proliferation (58). In addition, injection of noradrenaline causes an increase in proliferation, while blocking noradrenaline pharmacologically causes a decrease in cell proliferation (58). Treatment with cytotoxic drugs

initially results in a decrease in cell proliferation (22,23). Depending upon the extent of cell damage or death following cytotoxic drug treatment, a transient surge in cell proliferation occurs which repopulates the crypt (22,23,60). The time needed for restoration of the crypt population is probably dependent upon the number of viable cells in the crypt that are capable of dividing. Most of the above factors that influence the rate of cell proliferation are present in the normal animal and probably interact to balance the loss of cells from the surface of the crypts which maintains a steady state population.

C. Cell Migration

In addition to cell proliferation and cell sloughing, the rate of cell migration from the lower portions of the crypt is also important in maintaining normal crypt dynamics. Presently, there is poor agreement with regard to the rate of cell migration in the crypts of normal mouse colon (2,44,45,56). Two mechanisms have been proposed to explain the migration of cells upward in the intestinal crypts: (1) the pressure exerted by mitotically-active cells in the base of the crypts (6,11,12,45,48) or (2) the amoeboid migration of cells along the crypt walls (15,36).

Cells have been shown to migrate *in vitro* by cytoplasmic streaming and extension of surface protrusions that form lopopodia (55). A similar amoeboid movement of cells in vivo is observed during embryonic development and during migration of white blood elements peripheral tissues. Studies of cell adhesion to the adjacent substrata *in vitro*

indicate that this process does not appear to be a factor in the control of either the rates of cell migration or cell division (15). To what extent amoeboid movement contributes to the upward migration of cells in the intestinal crypts has not been clearly established.

Cell division in the intestinal crypts has been postulated by many investigators to force cells upward; however, this process has not been substantiated as the major contributing factor in cell migration. Recent reports in support of this theory have demonstrated that the cell migration rate increases as cell production increases, and that cells accelerate from the base of the crypt to the upper limit of the proliferative zone where they migrate at a constant rate until they are sloughed from the crypt surface (11,12,56).

The information is unclear whether the rate of cell migration of the epithelium is dependent on the concurrent migration of the cellular elements of the underlying fibroblast sheath. Pascal et al. (39) reported that epithelial cells in the crypts migrate in unison with the pericryptal fibroblasts in the lamina propria suggesting that some intimate contact or relationship existed between the two populations of cells. More recently it has been reported that concurrent migration of elements of the pericryptal fibrblast sheath did not occur (38).

The normal rates of crypt cell migration in the mouse colon have been estimated to range from 0.14 to 0.70 cell positions per hour (2,44,46,56). These variations in rates of migration may result from the differences in methods of analysis. Migration rates were estimated

from the total turnover of labeled cells after a single injection of ^{3}H-thymidine (56), or from the changes in the mean position of the highest labeled cells during a specified time period following labeling (2,44,46).

The information concerning the effects of cytotoxic drug treatment or irradiation on the migration of intestinal epithelial cells is conflicting. Hennings and Devik (26) reported that cells in the small intestine did not migrate as far up the intestinal villi after extended exposure to hydroxyurea, while Altmann (1) reported that migration of villous cells in the small intestine continued unaffected during treatment with methotrexate. Both of these drugs act by inhibiting DNA synthesis. In addition, reports of the effects of irradiation on cell migration range from continued migration without any effects (1) to migration of cells at reduced rates (36). These differing views may reflect the experimental procedures that were used, but it appears that further studies using cytotoxic agents under carefully planned experimental protocols may yield information regarding the role of cell division in the migration of intestinal cells.

D. Colon Cancer in Mice Treated with 1,2-Dimethylhydrazine

Lacquer (31) in 1963 first induced intestinal cancer by feeding rodents ground cycad nuts (Cycas circinalis L.) mixed with laboratory chow. Cycasin is the glycoside isolated for the cycad nuts and contains the active carcinogen, methylazoxymethanol (61). 1,2-Dimethylhydrazine is produced commercially and has been shown to be specific for the induction of tumors in the distal colon of laboratory animals (17,54).

DMH is converted to the same active carcinogen, methylazoxymethanol, via a series of metabolic steps in the liver. The activated carcinogen is secreted from the liver as a conjugate of a bile acid and is freed by bacterial enzymes in the lumen of the colon (61). The tumors which are experimentally produced in laboratory animals closely resemble adenocarcinoma of the colon in humans; therefore, the preneoplastic stages of carcinogenic transformation can be analyzed during progressive treatments before frank tumors appear. Microscopic preneoplastic lesions, often referred to as focal atypias, appear as early as 8 weeks after treatment and grossly visible adenocarcinoma of the colon occurs as early as 20 weeks (9,17,54). The number of animals developing tumors, the tumor yield per animal and the latency period before the appearance of tumors are dependent upon dosage and route of administration (18,28). Oral, intragastric, intrarectal and subcutaneous routes of administration of DMH all produce tumors in the colon of rodents, yet the subcutaneous route seems to be the most effective as well as convenient (28). The commonly used dosage of 20 mg/kg body wt. via subcutaneous injection consistently produces tumors after 20 weekly treatments without toxic contraindications (41,54).

The mucosa of the descending colon may be unique in its susceptibility to tumor induction. Transposition of the descending colon segments to the small intestine result in tumor formation in the transposed colon segment (7,21). Similarly, segments of the small

intestine transposed to the descending colon do not develop tumors after chronic treatment with DMH, while tumors develop consistently in the adjacent colonic mucosa (7,21). This indicates mucosal factors are of more importance in carcinogenesis than the environment of the lumen. Although the presence of bile acids enhance the proliferation of colonic cells and the absence of bile causes a reduction in labeling and mitotic indices (19), biliary excretion of activated carcinogens may not be essential for tumor induction in the colon. There are currently conflicting reports whether tumors will develop in an isolated distal segment of the colon after experimental colostomy in DMH treated animals (35,37). If tumors develop in a colon segment isolated from luminal contents, it would suggest that a systemic dissemination of the carcinogen occurs as well as through the excretion of bile salts.

Many previous studies of normal cell kinetics in the intestinal epithelium provide a detailed background necessary to assess the alterations in cell kinetics which occur during the early stages of chemically-induced carcinogenesis (11,12,34,36,42). In addition, ultrastructural changes in the membranes of colonic epithelial cells have been reported to occur in hyperplastic and adenomatous human colonic mucosa (30).

Several factors that modify the efficiency of tumor induction in DMH treated animals have been identified. Animals treated concurrently with DMH and selenium or butylated hydroxytoluene have a decreased tumor incidence (13,29), while administration of bile acids and

bacterially-induced crypt hyperplasia enhance the effects of DMH carcinogenesis (3,14,19). In experimental tumors of the colon there are many specific biochemical (5,20,41,43,47,49) and cell kinetic changes that are thought to precede and accompany neoplasia (18,33,44,45,54,59). Finally, an increase in the total number of cells in dilated and elongated crypts, and an increase in the proliferative population of cells in the crypts serve to alter the mucosa and these changes may promote the development of preneoplastic lesions (9,18,44,45,52,54,59).

BIBLIOGRAPHY

1. Altmann, G.G. 1974 Changes in the Mucosa of the Small Intestine Following Methotrexate Administration or Abdominal X-Irradiation. Am. J. Anat. 140: 263-280.

2. Barthold, S.W. 1979 Autoradiographic Cytokinetics of Colonic Mucosal Hyperplasia in Mice. 1979 Cancer Res. 39: 24-29.

3. Barthold, S.W. 1981 Relationship of Colonic Mucosal Background to Neoplatic Proliferative Activity in Dimethylhydrazine-treated Mice. Cancer Res. 41: 2616-2620.

4. Bizzozero, G. 1888 Ueber die regeneration der elementé der schlauchformigen drusen und des epithels des magendorum kanals. Anat. Anz. 3: 781.

5. Brambilla, G., M. Cavanna, S. Parodi, L. Sciaba, A. Pino and L. Robbiano. 1978 DNA Damage in Liver, Colon, Stomach, Lung and Kidney of BALB/c Mice Treated with 1,2-Dimethylhydrazine. Int. J. Cancer 22: 174-180.

6. Cairnie, A.G., L.F. Lamerton, G.G. Steel. 1965 Cell Proliferation Studies in the Intestinal Epithelium of the Rat. I. Determination of the Kinetic Parameters. Exp. Cell Res. 39: 528-538.

7. Celik, C., A. Mittleman, N.S. Paolini, D. Lewis and J.T. Stevens. 1981 Effects of 1,2-Symmetrical Dimethylhydrazine on Jejunocolic Transposition in Sprague-Dawley Rats. Cancer Res. 41: 2908-2911.

8. Change, W.W.L. 1971 Renewal of the Epithelium in the Descending Colon of the Mouse: III. Diurnal Variation in the Proliferative Activity of Epithelial Cells. Am. J. Anat 131: 111-120.

9. Chang, W.W.L. 1978 Histogenesis of Symmetrical 1,2-Dimethylhydrazine-Induced Neoplasms of the Colon in the Mouse. J. Natl. Cancer Inst. 60: 1405-1418.

10. Chang, W.W.L. 1981 Degenerative Behavior of Epithelial cells in the Colonic Crypt of the Mouse Following Administration of Colonic Carcinogen 1,2-Dimethylhydrazine. Cancer Lett. 13: 111-118.

11. Chang, W.W.L., and C.P. Leblond. 1971 Renewal of the Epithelium in the Descending Colon of the Mouse. I. Presence of Three Populations: Vacuolated-Columnar, Mucous and Argentaffin. Am. J. Anat. 131: 73-100.

12. Chang, W.W.L., and J.J. Nadler. 1975 Renewal of the Epithelium in the Descending Colon of the Mouse. IV. Cell Population Kinetics of Vacuolated-Columnar and Mucous Cells. Am. J. Anat. 144: 39-56.

13. Clapp, N.K., N.D. Bowles, L.C. Satterfield and W.C. Klima. 1979 Selective Protective Effect of Butylated Hydroxytoluene Against 1,2-Dimethylhydrazine Carcinogenesis in BALB/c Mice. J. Natl. Cancer Inst. 63: 1081-1085.

14. Cohen, B.I., and R.F. Raicht. 1981 Effects of Bile Acids on Colon Carcinogenesis in Rats Treated with Carcinogens. Cancer Res. 41: 3759-3760.

15. Curtis, A.S.G., and T.E.J. Buultjens. 1973 Cell Adhesion and Locomotion. In Locomotion of Tissue Cells, Ciba Foundation Symposium 14. Associated Scientific Publishers: New York, NY, 171-194.

16. de Rodriguez, M.S.B., J.P. Sunter, A.J. Watson, N.A. Wright and D. R. Appleton. 1979 Cell Population Kinetics in the Mucosal Crypts of the Descending Colon of the Mouse. Virchows Arch. B. Cell Path. 29: 351-361.

17. Deschner, E.E. 1974 Experimentally Induced Cancer of the Colon. Cancer Res. 34: 824-828.

18. Deschner, E.E., F.C. Long and A.P. Maskens. 1979 Relationship Between Dose, Time and Tumor Yield in Mouse Dimethylhydrazine-Induced Colon Tumorigenesis. Cancer Lett. 8: 23-28.

19. Deschner, E.E. and R.F. Raicht. 1979 Influence of Bile on the Kinetic Behavior of Colonic Epithelial Cells of the Rat. Digestion 19: 322-327.

20. Filipe, M.I. and A.C. Branfoot. 1974 Abnormal Patterns of Mucus Secretion in Apparently Normal Mucosa of the Large Intestine with Carcinoma. Cancer 34: 282-290.

21. Gennaro, A.R., R. Villaneuva, Y. Sukonthaman, V. Vathanophas and G.P. Rosemond. 1973 Chemical Carcinogens in Transposed Intestinal Segments. Cancer Res. 33: 536-541.

22. Hagemann, R.F. and S. Lesher. 1971 Irradiation of the G.I. Tract: Compensatory Response of Stomach, Jejunum and Colon. Brit. J. Rad. 44: 599-602.

23. Hagemann, R.F. and S. Lesher. 1971 Intestinal Crypt Survival and Total and Per Crypt Levels of Proliferative Cellularity Following Irradiation: Age Response and Animal Lethality. Radiation Res. 47: 159-167.

24. Hagemann, R.F. and J.J. Stragand. 1977 Fasting and Refeeding: Cell Kinetic Response of Jejunum, Ileum and Colon. Cell Tissue Kinetics 10: 3-14.

25. Hamilton, E. 1979 Diurnal Variation in Proliferative Compartments and their Relation to Cryptogenic Cells in the Mouse Colon. Cell Tissue Kinetics 12: 91-100.

26. Hennings, H., and F. Devik. 1971 Comparison of Cytotoxicity of Hydroxyurea in Normal and Rapidly Proliferating Epidermis and Small Intestine in Mice. Cancer Res. 31: 277-282.

27. Hoff, M.B., W.W.L. Chang and K.M. Mak. 1981 Effect of Estrogen on Cell Proliferation in the Colonic Mucosa of the Mouse. Virchows Arch. B. Cell Path. 35: 263-273.

28. Izumi, K., H. Otsuka and K. Furuya. 1980 Carcinogenicity of 1,2-Dimethylhydrazine Dihydrochloride in Mice. Tokushima J. Exp. Med. 27: 5-13.

29. Jacobs, M.M., C.F. Forst and F.A. Beams. 1981 Biochemical and Clinical Effects of Selenium on Dimethylhydrazine Induced Colon Cancer in Rats. Cancer Res. 41: 4458-4465.

30. Kaye, G.I., C.M. Fenoglio, R.R. Pascal and N. Lane. 1973 Comparative Electron Microscopic Features of Normal, Hyperplastic and Adenomatous Human Colonic Epithelium. Gastroenterology 64: 926-945.

31. Laqueur, G.L., O. Mickelsen, M.G. Whiting and L.T. Kurland. 1963 Carcinogenic Properties of Nuts from Cycas Circinalis L. Indigenous to Guam. J. Natl. Cancer Inst. 31: 919-933.

32. Leblond, C.P. and C.E. Stevens. 1948 The Constant Renewal of the Intestinal Epithelium in the Albino Rat. Anat. Rec. 100: 357-378.

33. Lipkin, M. 1974 Phase 1 and Phase 2 Proliferative Lesions of Colonic Epithelial Cells in Diseases Leading to Colonic Cancer. Cancer 34: 878-888.

34. Lipkin, M., and H. Quastler. 1962 Cell Population Kinetics in the Colon of the Mouse. J. Clin. Invest. 41: 141-146.

35. McMinn, A.R. and T.C. Richards. 1981 Induction of Tumors with 1,2-Dimethylhydrazine in Segments of Descending Colon of Mice Following a Mid-Left Colostomy. Proc. Amer. Assoc. Cancer Res. 22:60.

36. Messier, B., and C.P. Leblond. 1960 Cell Proliferation and Migration as Revealed by Radioautography after Injection of ^{3}H-Thymidine into Male Rats and Mice. Am. J. Anat. 106: 247-285.

37. Navarette, A. and H.J. Spjut. 1967 Effect of Colostomy on Experimentally Produced Neoplasms of the Colon of the Rat. Cancer 20: 1466-1472.

38. Neal, J.V. and C.S. Potten. 1981 Description and Basic Cell Kinetics of the Murine Pericryptal Fibroblast Sheath. Gut 22: 19-24.

39. Pascal, R.R., G.I. Kaye and N. Lane. 1968 Colonic Pericryptal Fibroblast Sheath: Replication, Migration and Cytodifferentiation of a Mesenchymal Cell System in Adult Tissue. I. Autoradiographic Studies of Normal Rabbit Colon. Gastroenterology 54: 835-851.

40. Potten, C.S., and L. Kovacs. 1973 An Estimation of Proliferative Population Size in Stomach, Jejunum and Colon of DBA-2 Mice. Cell Tissue Kin. 6: 125-134.

41. Pozharriski, K.M., Y.M. Kapustin, A.J. Likhachev and J.D. Shaposhnikov. 1975 The Mechanims of Carcinogenic Action of 1,2-Dimethylhydrazine (SDMH) in Rats. Int. J. Cancer 15: 673-683.

42. Quastler, H., and F.G. Sherman. 1959 Cell Population Kinetics of the Intestinal Epithelium of the Mouse. Exp. Cell Res. 17: 420-438.

43. Rana, R.S., R.H. Stevens, L. Oberley, D.P. Loven, J.M. Graves, D.A. Cole and E.S. Meek. 1980 Evidence for a Defective Mitochondrial Membrane in 1,2-Dimethylhydrazine-Induced Colon Adenocarcinoma in Rat: Enhanced Lipid Peroxidation Potential in vitro. Cancer Lett. 9: 237-244.

44. Richards, T.C. 1977 Early Changes in the Dynamics of Crypt Cell Populations in Mouse Colon following Administration of 1,2-Dimethylhydrazine. Cancer Res. 37: 1680-1685.

45. Richards, T.C. 1981 The Effects of the Carcinogen, 1,2,-Dimethylhydrazine, on Turnover of ^{3}H-Thymidine Labeled Cells from Mucosal Glands of Mouse Colon. Anat. Rec. 200: 299-308.

46. Rijke, R.P.C., W. van der Meer-Fieggen and H. Galjaard. 1974 Effects of Villus Length of Cell Migration in Small Intestinal Epithelium. Cell Tissue Kin. 7: 577-586.

47. Salser, J.S., W.J. Ball and M.E. Balis. 1976 Biochemical Changes in Premalignant Intestines. Cancer Res. 36: 3495-3498.

48. Sawicki, W., J. Rowinski, W. Maciejewski and K. Kwarecki. 1968 Kinetics of Proliferation and Migration of Epithelial Cells in the Guinea Pig Colon. Exp. Cell Res. 50: 93-103.

49. Stevens, R.H., D.P. Loven, J.W. Osborne, J. P. Prall and A.J. Lawson. 1977 Cyclic Nucleotide Concentrations in 1,2-Dimethylhydrazine Induced Rat Colon Adenocarcinoma. Cancer Lett. 4: 27-33.

50. Stragand, J.J. and R.F. Hagemann. 1977 Effect of Luminal Contents on Colonic Cell Replacement. Am. J. Physiol. 233: 208-211.

51. Sunter, J.P., N.A. Wright and D.R. Appleton. 1978 Cell Population Kinetics in the Epithelium of the Colon of the Male Rat. Virchows Arch B. Cell Path. 26: 275-287.

52. Sunter, J.P., A.J. Watson and D.R. Appleton. 1981 Kinetics of the Non-Neoplastic Mucosa of the Large Bowel of Dimethylhydrazine-Treated Rats. Br. J. Cancer 44: 35-44.

53. Thrasher, J.D. 1967 Age and the Cell Cycle of the Mouse Colonic Epithelium. Anat. Rec. 157: 621-626.

54. Thurnherr, N., E.E. Deschner, E.H. Stonehill, and M. Lipkin. 1973 Induction of Adenocarcinomas of the Colon in Mice by Weekly Injections of 1,2-Dimethylhydrazine. Cancer Res. 33: 940-945.

55. Trinkans, J.P. 1973 Modes of Cell Locomotion in vivo. In Locomotion of Tissue Cells, Ciba Foundation Symposium 14. Associated Scientific Publishers: New York, NY. 233-249.

56. Tsubouchi, S. 1981 Kinetic Analysis of Epithelial Cell Migration in the Mouse Descending Colon. Am. J. Anat. 161: 239-246.

57. Tsubouchi, S. and C.P. Leblond. 1979 Migration and Turnover of Entero-Endocrine and Caveolated Cells in the Epithelium of the Descending Colon, as shown by Radioautography after Continuous Infusion of ^{3}H-thymidine into Mice. Am. J. Anat. 156: 431-452.

58. Tutton, P.J.M. 1977 Neural and Endocrine Control Systems Acting on the Population Kinetics of the Intestinal Epithelium. Med. Biol. 55: 201-208.

59. Tutton, P.J.M. and D.H. Barkla. 1976 Cell Proliferation in the Descending Colon of Dimethylhydrazine Treated Rats and in Dimethylhydrazine-Induced Adenocarcinomata. Vircows Arch. B. Cell Path. 21: 147-160.

60. Tutton, P.J.M., and D.H. Barkla. 1978 Cell Proliferation in Dimethylhydrazine-Induced Colonic Adenocarcinomata Following Cytotoxic Drug Treatment. Virchows Arch. B. Cell Path. 28: 151-156.

61. Weisburger, J.H. 1971 Colon Carcinogens: Their Metabolism and Mode of Action. Cancer 28: 60-70.

II. Statement of Problem

Control of the rates of cell migration in the crypts of the descending colon is important in maintaining the crypt population in a steady state. Upward migration of cells may be due to the constant division of cells in the proliferative zone of the crypts, and the speed at which cells migrate should directly correlate with the size of the population of dividing cells because each cell division adds to the total rate of cell movement (2,5). Previous studies have shown that the rate of cell migration increases as the mitotic activity in the crypt population increases (1,3,4). Weekly treatment of mice with the carcinogen, 1,2-Dimethylhydrazine (DMH), causes an enlargement of the crypts, with progressive transformation of cells that results in the appearance of tumors after 20 weeks of treatment. It is not known if alterations in the rates of cell migration and cell loss from the crypts play a role in the formation of tumors in the colon. The purpose of these studies were to identify some of the underlying mechanisms involved in crypt cell migration and to determine if the rate of cell migration and cell loss are changed in crypts altered by treatment with DMH.

The following studies were performed to determine: (1) the rates of crypt cell migration in control mice and mice treated progressively with DMH, (2) the effects of mitotic inhibition on cell migration in mice treated with and without DMH and (3) whether there are changes in the rate of crypt cell loss in mice treated progressively with DMH.

The studies described above were performed by light microscopic radioautography using ^{3}H-thymidine as a precursor in DNA synthesis and are presented in the Manuscript of this thesis. Preliminary experiments using vinblastine to inhibit mitotic cells in the crypts are presented in the Appendix.

Bibliography

1. Barthold, S.W. 1979 Autoradiographic Cytokinetics of Colonic Mucosal Hyperplasia in Mice. 1979 Cancer Res. 39: 24-29.

2. Chang, W.W.L. 1978 Histogenesis of Symmetrical 1,2-Dimethylhydrazine Induced Neoplasms of the Colon in the Mouse. J. Natl. Cancer Inst. 60: 1405-1418.

3. Richards, T.C. 1977 Early Changes in the Dynamics of Crypt Cell Populations in Mouse Colon following Administration of 1,2-Dimethylhydrazine. Cancer Res. 37: 1680-1685.

4. Rijke, R.P.C., W. van der Meer-Fieggen and H. Galjaard. 1974 Effects of Villus Length on Cell Migration in Small Intestinal Epithelium. Cell Tissue Kin. 7: 577-586.

5. Tsubouchi, S. 1981 Kinetic Analysis of Epithelial Cell Migration in the Mouse Descending Colon. Am. J. Anat. 161: 239-246.

III. MANUSCRIPT

Changes in Cell Migration and the Rate of Cell Loss from Crypts in the Descending Colon of Mice Treated with 1,2-Dimethylhydrazine.

Abstract

The rates of cell migration and cell loss in the crypts of the mouse colon are factors which contribute to the maintenance of a steady state, and treatment with the carcinogen, 1,2-Dimethylhydrazine (DMH), alters this dynamic balance. This study was designed to determine how these indices were modified by treatment with DMH and if these changes underlie the development of neoplastic lesions.

Female Swiss Webster mice were injected weekly, s.c., with DMH (20 mg/kg body wt.) for 1,2,4,8,12,16 or 20 weeks. In the first experiment, animals were given a pulse label of ^{3}H-thymidine, treated with hydroxyurea for 12 hours (3-15 hours after ^{3}H-thymidine) and sacrificed 3,15 and 27 hours after ^{3}H-thymidine. In the second experiment, animals were given four injections of ^{3}H-thymidine over 12 hours, then sacrificed 3,3 1/2,4,5,7 and 10 days later. Rates of cell migration using light microscopic radioautography were determined from the change in position of the highest labeled cells in the crypts. The rate of cell loss was measured from the decrease in crypt labeling index from 3 to 10 days.

The rate of cell migration in controls was 0.26 cells/hour, and this rate increased as the duration of DMH treatment increased reaching a maximum of 0.52 cells/hour after 20 weeks. Cell migration in both control and DMH treated mice was inhibited by hydroxyurea. Although the relative rate of cell loss increased in all DMH treatment groups, this

rate did not vary as a function of treatment duration. However, since the zone of proliferation in the crypts progressively enlarged, the absolute number of cells produced and subsequently lost increased as the duration of DMH treatment increased.

It could not be concluded from this study that changes in cell loss and cell migration correlated with the appearance of neoplastic lesions in the colon crypts.

INTRODUCTION

Cells in the crypts of the descending colon represent a population in a steady state. The loss of cells from the epithelium at the luminal surface is balanced by mitotic activity in the base of the crypts. In addition to mitosis and sloughing, cell migration from the lower portions of the crypt is also important in crypt dynamics, since the rate and distance cells migrate are factors in maintaining crypt size. Presently there is poor agreement with regard to the rate of cell migration in the crypts of normal mouse colon (2,22,23,34). Two mechanisms have been proposed to explain the migration of cells upward in the intestinal crypts: (1) the pressure exerted by mitotically active cells in the base of the crypts (3,7,8,23,27); or (2) the amoeboid migration of cells along the crypt walls (9,19).

If pressure resulting from mitotic activity in the base of the crypt is primarily responsible for cell migration, the rate of migration should accelerate from the base of the crypt to the upper limit of the proliferative zone and thereafter remain constant until cells reach the crypt surface (3,8,22,27,34). Alternatively, if cell migration is due primarily to amoeboid movement the rate of cell migration should be constant throughout the length of the crypt. Altmann (1) and Sherman and Quastler (29) reported that cells in the intestinal epithelium continued to migrate after exposure to irradiation sufficient to kill mitotic cells. Altmann (1) also reported that crypt cell migration in the small intestine continued during daily treatment with methotrexate,

an inhibitor of nucleic acid synthesis. These findings suggest that cell proliferation may not be a critical factor for the migration of crypt cells. Conversely, if migration of crypt cells is dependent upon the mitotic activity in the base of the intestinal crypts, then blockage of mitosis experimentally should result in a cessation of cell migration. In support of this hypothesis, Hennings and Devik (12) reported that villous cells in the small intestine of mice treated with hydroxyurea had not migrated as far up the villi as in control animals suggesting that inhibition of mitosis stopped cell migration.

Weekly treatments with the carcinogen, 1,2-dimethylhydrazine (DMH), result in a lengthening of the crypts and an increased population of proliferative cells (10,33) leading to the establishment of a new steady state. The cells have been shown to migrate faster within the enlarged crypts of DMH treated mice (22,23). Since the rate at which cells migrate in the crypts is one of the factors controlling the lifespan and turnover of the epithelium, an increased rate of cell migration may be a mechanism by which the dynamics of enlarged crypts are modified in response to an increased mitotic activity.

Cells are continously sloughed from the crypt surface at a rate equal to the cell birth in the base of the crypts to maintain the population in a steady state. The increased crypt length in mice treated with DMH may be due to the cells not being sloughed from the luminal surface. Lipkin (14) suggested that cells migrating to the

crypt surface in DMH-treated animals were less mature and subsequently retained at or near the crypt surface. It was also suggested that polyps and adenocarcinoma may eventually develop from these retained cells. Richards (23) reported that the labeled progeny of stem cells in the lower crypt segments appear to be retained in the crypts longer after treatment with DMH; however, the fate of these cells in the upper portions of the crypt was not followed. A study of the rate of cell loss from upper crypt segments of mice treated progressively with DMH should determine whether cell loss is altered by treatment with DMH.

The present experiments using radioautography were undertaken to determine: (1) the rate of normal crypt cell migration in the mouse descending colon, (2) if inhibition of mitotic activity with hydroxyurea would affect the migration of crypt cells, (3) if treatment of mice with DMH alters the rate of crypt cell migration and if inhibition of mitosis would also affect this altered rate of cell migration and (4) the rate that cells are lost from the non-proliferative region of the colon crypts in mice treated with DMH compared to normal animals.

MATERIALS AND METHODS

I. Preliminary Treatments and Tissue Preparation for Radioautography

Two experiments were performed and those treatments that were common to both are described here, while those details unique to each experiment are given below in separate protocols.

Virgin female Swiss Webster mice (20-25 grams body wt.) were housed 6 animals per cage, maintained at 22°C with a 12 hour light/dark cycle (0700-1900 hours) and were given standard laboratory mouse chow (Oregon State University; Corvallis, OR) and water ad libitum. One half the animals were treated with weekly subcutaneous (s.c.) injections of 1,2-dimethylhydrazine-hydrochloride (DMH; American Scientific Co.; Portland, OR) at 20 mg/kg body wt. for periods of 1,2,4,8,12,16 or 20 weeks. DMH was dissolved in 0.001M EDTA and buffered with $NaHCO_3$ (pH=7). The other half of the animals served as controls and were given equal volumes of buffered 0.001M EDTA at the same time intervals.

Animals were sacrificed by cervical dislocation. The abdomen was opened and a 1 cm segment of descending colon at the level of the pelvic rim was removed, rinsed in ice cold normal saline and fixed in a phosphate buffered 4% formaldehyde-1% glutaraldehyde solution. The tissue was dehydrated in a graded series of ethanol before being embedded in glycol methacrylate (Polysciences Inc.; Warrington, PA). For histologic examination, 2 m transverse sections of colon were mounted on glass slides and stained with hematoxylin (32). Three m sections of colon were prestained by hydrolysis of nucleic acids in

1N HCl at 60°C for 10 minutes followed by staining with Feulgen reaction. Slides were coated with Kodak NTB-3 (Eastman Kodak; Rochester, NY) emulsion (diluted 2:1 with distilled water) at 40°C, dried overnight, placed in a leaded box with a Drierite (W.A.H. Drierite Co.; Xenia, OH) desiccant and exposed for 3 or 4 weeks. Slides were developed at 18°C with Kodak Dektol (1 minute), fixed in Kodak Fixer (2 minutes) and rinsed in filtered water (1 hour) (26). Slides were air dried and mounted with coverslips for observation.

II. Experimental Protocols

A. Determination of Rates of Crypt Cell Migration: (Experimental Protocol I; Figure Ia).

One week following the termination of DMH treatment, animals from the DMH and control groups were divided into the treatment subgroups designated: DMH+HU, DMH only, hydroxyurea only (HU; Squibb Inc.; Princeton, NJ), and untreated controls with 9 animals per subgroup. All animals were given a single intraperitoneal (i.p.) injection of ^{3}H-thymidine at 0.5 Ci/g body wt. (specific activity, 20 Ci/mmole; New England Nuclear; Boston, MA) at 0800 hours. The subgroups receiving HU (200 mg/ml in normal saline) were given an initial i.p. injection (2.0 mg/kg body wt.) at 1100 hours and repeated at 2 hour intervals for 12 hours. The DMH only and control subgroups were injected i.p. with equal volumes of normal saline at the same time intervals. Three animals from each of the four subgroups were sacrificed at 1100 hours

(3 hours after ^{3}H-thymidine), at 2300 hours (15 hours after ^{3}H-thymidine) and 1100 hours the following day (27 hours after ^{3}H-thymidine).

Crypts selected for analysis met the following criteria: (1) open at the lumen, (2) in contact with the muscularis mucosa at the base of the crypt and (3) a single longitudinal column of cells extending from the base to the surface of the crypt (29). Cells were considered labeled if there were a minimum of three silver grains over the nucleus.

Forty crypt columns per animal were counted; the total number of nuclei per crypt column and the number and position of labeled nuclei were recorded. Mean crypt length, labeling index (LI=labeled nuclei/total nuclei per crypt column X 100) and position of the highest labeled cells for each animal were determined and the means computed for each subgroup.

The migration rate of crypt cells (cell positions per hour) was estimated from the change in the mean position of the highest labeled cell at the intervals specified after ^{3}H-thymidine labeling. The change in the mean position of the highest labeled cell was determined by counting the change in cell position relative to both the base and the surface of the crypts. Differences in the rate of cell migration between the HU treated and the non-HU treated animals were compared as were those between animals treated with DMH and untreated controls.

The size of the proliferative and non-proliferative zones in the crypts of control and DMH treated animals were estimated from the

position of the mean highest labeled cell 3 hours after ^{3}H-thymidine. The position of the highest labeled cell marked the upper limit of the zone of proliferation in the crypt below which cells were capable of incorporating ^{3}H-thymidine into DNA. The number of cells from the highest labeled cell to the crypt surface represented the size of the non-proliferative zone in the crypt.

B. Loss of Labeled Cells from the Non-Proliferative Zone of Crypts (Experimental Protocol II; Figure 1b)

One week following the termination of DMH treatment, all animals were given four i.p. injections of ^{3}H-thymidine (0.25 Ci/g body wt.) at 0900, 1300, 1700 and 2100 hours to label a maximum number of crypt cells for determining cell loss over 10 days. Three days later at 0900 hours the animals were divided into subgroups designated: DMH+HU, DMH only, HU only and controls. The animals in the subgroups were treated with HU or saline for 12 hours as previously described. Three animals from each subgroup were sacrificed 3,3 1/2,4,5,7 and 10 days after the initial labeling with ^{3}H-thymidine.

Twenty crypt columns per animal (selection criteria described above) were counted; the total number of nuclei per crypt column and the total number labeled nuclei in the non-proliferative zone of crypt columns were recorded. Since the purpose of this study was to follow post-mitotic cells that had migrated toward the crypt surface, a more rigid criteria for inclusion as a labeled cell was used. To exclude

lightly labeled daughter cells originating from heavily labeled cells in the crypt base, each cell had to have a minimum of 8-10 silver grains over the nucleus to be included in the cell count. For this reason, the number of labeled nuclei included in the analysis represented only a portion of the total number of labeled nuclei that were present. The LI of the non-proliferative zone was determined from the percentage of heavily labeled nuclei in the non-proliferative zone.

The rate of decrease of LI that represented the loss of labeled cells as they migrated from the non-proliferative region of the crypts was compared between animals of the four subgroups. Since normal cells reach the crypt surface 3 to 4 days after birth in the crypt base, the loss of the labeled cells was monitored from 3 to 10 days after ^{3}H-thymidine.

III. Statistical Analysis

Statistical significance of differences in the rates of crypt cell migration (Protocol I) in each of the four treatment subgroups and the size of the functional zones in the crypts during progressive DMH treatments were determined by analysis of variance (ANOVA). Statistical analyses for comparison of the loss of labeled cells (Protocol II) from the crypts of DMH treated and control animals were determined with a split plot or nested ANOVA to evaluate for: (1) the main effects of DMH treatment, (2) 2-way interactions to compare the rate of decrease in LI

for the control and DMH groups during the course of treatment and (3) 3-way interactions comparing the decreasing LI for DMH and control treatments by week by day (30).

RESULTS

The dynamics of cell populations in the crypts of descending colon in mice were altered by progressive weekly treatments with DMH. The mean crypt length increased from 28.9±0.3 cells in the control group to 46.5±1.4 in the animals treated with DMH for 20 weeks, and the LI of the crypts increased from 13.5±0.9 in the controls to 17.3±2.5 in the animals treated with DMH for 20 weeks (Table I). The number of cells in the proliferative zone increased as the number of weekly treatments with DMH increased, while the number of cells in the non-proliferative zone did not change significantly during the course of DMH treatment (Figure II). As a result, the proliferative fraction of the crypt increased as the effect of treatment with DMH progressed.

I. Determination of Crypt Cell Migration:

A. Normal Crypt Cell Migration

Cell migration in the crypts was determined from the changes in the highest position of labeled cells with time. The relative change in the highest mean position of labeled cells indicated that crypt cells migrated at 0.28 cells/hour between 1100 and 2300 hours and 0.24 cells/hour between 2300 and 1100 hours the following day (Table II).

B. Crypt Cell Migration after Treatment with Hydroxyurea

Crypt cell migration in the mouse descending colon was inhibited during the 12 hours of HU treatment when cell division was blocked

(Table II). During the 12 hour recovery period following HU treatment (2300-1100 hours) crypt cells resumed migration at a rate of 0.18 cells/hour. This was significantly greater than the rate of cell migration during the 12 hours of HU treatment ($p<.001$) but less than the rate of cell migration in control animals.

C. Effects of DMH Treatment on Crypt Cell Migration

The rate of crypt cell migration in mice treated with DMH increased above control values (Table I), and this increase directly correlated with the number of weekly DMH treatments ($r=+0.87$, $N=42$). Rates of crypt cell migration in the groups of mice treated with DMH for 1,2 and 4 weeks were not different from controls. The rates of cell migration were significantly elevated above control values after 8 weeks of treatment, and the maximum rate of cell migration (0.52 cells/hour) was observed in the animals treated with DMH for 20 weeks.

D. Effects of Hydroxyurea Treatment on Crypt Cell Migration in DMH Treated Mice

Crypt cell migration was inhibited with HU in the animals treated with DMH. Progressive treatments with DMH had no effect on the response of cells to inhibition with HU (Table II). However, recovery from treatment with HU was apparently slower in the DMH treated animals, because cell migration did not resume at a rate comparable to that observed in the animals treated with HU only.

II. Effects of DMH Treatment on Crypt Cell Loss

To determine the dynamics of cell loss from the crypts in the descending colon of mice treated with DMH, the loss of labeled cells from the non-proliferative zone of crypts was assessed. Three days were allowed after ^{3}H-thymidine for labeled cells to migrate into the non-proliferative zone of the crypt. The following changes in the rate of crypt cell loss in DMH treated animals compared to those observed in the control animals were: (1) rate of crypt cell loss in the DMH treated animals was significantly greater than in the control animals regardless of the length of DMH treatment ($p<.01$) (Figure III) and (2) the increased rate of cell loss was the same for all DMH treated groups. Because the relative rates of crypt cell loss were the same in all DMH treatment weeks, the data were combined for statistical analysis (Table II). Three and 3 1/2 days after ^{3}H-thymidine the LI of the non-proliferative zone in the DMH treated animals was significantly greater than in the control groups. Subsequently, the LI in the DMH treated animals rapidly decreased and was not different from control groups on days 4 through 10.

Inhibition of mitotic activity in the crypts with HU did not alter the rate of cell loss in either DMH treated or control animals once the labeled cells had reached the crypt surface ($p<.05$). Although cell migration was shown to be inhibited during the 12 hours of HU treatment described above, the effects of inhibition 3 days later could not be detected by changes in the rate of cell loss from the crypts.

DISCUSSION

Results from this study define the rates of cell migration in the crypts of the descending colon of normal and DMH-treated mice and attempt to determine the possible mechanisms which control the migration of crypt cells. Cells migrate at 0.26 cells/hour in crypts of normal mice, and this rate increases progressively to 0.52 cells/hour in the crypts of animals treated with DMH for 20 weeks. In both these groups of animals, cell migration ceased after treatment with hydroxyurea (HU), a drug which inhibits DNA synthesis prior to cell division (36,37). Migration of cells resumed at a slower rate in all animals after HU treatment was terminated, but in animals treated with DMH the renewed rate of migration was less than in control animals. Weekly treatment with DMH results in an increase in the total number of crypt cells and a relatively larger proportion of these cells are mitotically active. Comparisons of mice treated with or without DMH demonstrate that the rate of cell loss from crypts is greater in animals treated with DMH.

The rate of normal crypt cell migration of 0.26 cells/hour in this study is in general agreement with some reports (34), but it is considerably higher than others (2,23). These discrepancies may stem from differences in methods used to estimate the rate of cell migration. In the present study rates of cell migration were determined from the changes in the position of the leading edge of labeled crypt cells at 3,15 and 27 hours after ^{3}H-thymidine (34). The current method is a more accurate estimate of cell migration, because changes in the

position of the cells are observed before any variation in the length of the cell cycle or diurnal fluctuations in the labeled cell populations occur. In addition, estimating the changes in position of labeled cells at 24,48,72 and 96 hours after ^{3}H-thymidine as performed in some studies (2,23) may be less accurate, because within this time period some labeled cells will divide and dilute the nuclear label and some labeled cells will have reached the crypt surface causing an underestimation in the rate of cell migration.

Hennings and Devik (12) reported that hydroxyurea (HU) inhibits cell migration in the small intestine. Conversely, Altmann (1) reported that daily treatment of mice with methotrexate, also an inhibitor of nucleic acid synthesis, has no effect on the migration of crypt and villous cells in the small intestine. Daily treatment with methotrexate may not have been frequent enough to halt cell division for 24 hours, since the physiologic removal of the drug is 90% complete in 12 hours (18). Therefore, the subsequent recovery and repopulation of the crypts was sufficient enough to obscure any inhibition of cell migration that did occur. Other studies have shown that cell migration in the small intestine either continues unaltered after irradiation (1) or continues at a reduced rate compared to controls (29). In any event, the above studies indicate that the mechanisms which control migration are not well understood.

The rate of cell migration in the intestinal crypts is postulated to be equal to the cumulative proliferative activity in the crypts (3,8,27). The basis for this assumption is that division of cells causes an acceleration in the rate of cell migration from the base of

the crypt to the upper limit of the proliferative zone, then cells migrate at a constant rate until they are sloughed from the luminal surface (34). The question that follows is: does cell migration in the colon crypts continue when cell division is blocked? If cell division is reponsible for cell migration, its disruption should result in a slower rate of cell migration. The results of this study demonstrate that migration of crypt cells in the mouse descending colon was inhibited during treatment with HU.

This finding suggested that further studies be performed to determine if the same relationship exists between cell migration and cell division in the crypts of mice treated chronically with DMH. The results demonstrate that the migration of crypt cells in DMH treated mice is also inhibited with HU, and the response of cells to mitotic inhibition did not change as the duration of DMH treatment increased. This suggests that factors controlling cell migration are not affected by carcinogenic transformation of the crypt population. However, following treatment with HU the resumption of cell migration in the DMH-treated animals was not as rapid as in control animals. Since the number of proliferative cells at risk to an S-phase cytotoxic agent is greatly increased in the DMH treated animals, the resumption of cell migration may have been delayed until the population of proliferative cells was restored. Any subsequent division of these cells would then initiate further cell migration in the crypts. Tutton and Barkla (35) reported a similar but more prolonged delay of 5 days in the recovery of crypt cells in DMH treated animals after exposure to 5-fluorouracil.

As the animals were progressively treated with DMH, the rate of cell migration in the crypts increased accordingly. The increased rates of cell migration in the animals treated with DMH for 20 weeks were approximately two-fold over control animals (22,23). If cell migration is dependent upon the cumulative proliferative activity in the crypts, then the increased population of proliferative cells in the DMH-treated animals would account for the rapid increase in the rate of crypt cell migration. This is confirmed by the significant increase in the rate of cell migration which occured after 8 weeks of DMH treatment, when crypts become hyperplastic due to the rapid expansion of the proliferative population of cells. However, this finding does not exclude the possibility that a similar increase in cell migration can also occur in response to conditions other than DMH treatment. Examples such as bacterially-induced crypt hyperplasia in the mouse colon (2) and crypt-villous hyperplasia in the small intestine of lactating rats (4) both have corresponding increases in the rate of cell migration. Similarly, the reduced crypt and villous populations that result from irradiation of the small intestine also coincide with a slower rate of cell migration (25).

As the crypt enlarges in response to DMH treatment, the proportion of mitotic cells in the crypt (proliferative fraction) enlarges beyond the fraction of 0.40 observed in controls (2,10,20,29,33,35). The size of the proliferative fraction in this study may have been slightly overestimated because the animals were sacrificed 3 hours after

^{3}H-thymidine rather than 1 hour (17) thus allowing labeled cells to migrate upward during the 3 hours. This time interval was necessary to the design of the experiment, because it allowed a portion of the labeled cells incorporating ^{3}H-thymidine to progress through S-phase and escape the effects of treatment with HU. The expansion of the crypt proliferative zone during the early weeks of DMH treatment may represent a transient compensatory response to the toxic effects of the carcinogen; however, the changes in crypts may be permanent after 8 weeks of treatment because hyperplasia persists after the termination of DMH treatment and a prolonged period of recovery (24). In these enlarged crypts, the rate of cell proliferation is higher due to the DMH-induced changes in the crypt cells, but a corresponding increase in the total number of post-mitotic or non-proliferative crypt cells does not occur.

The rate of cell migration is faster in animals treated with DMH, and the distance from the proliferative zone to the crypt surface does not change. Therefore, labeled cells reach the crypt surface in 1.7 days in the animals treated for 20 weeks compared to 3.0 days in control animals. Assuming that the process of post-mitotic maturation is not altered by DMH treatment, cells may either be less mature when they are sloughed from the crypt surface or they are not sloughed from the surface until they have matured a required period of time after their last division (8). If relatively immature cells can be sloughed from the mucosa after DMH, it would indicate that the carcinogen alters the crypt cells enabling them to be lost prematurely from the mucosa or that

DMH alters the underlying components of the basement membrane making cells less adherent. In either event, changes in the size of the proliferative pool of cells influence the rate of crypt cell migration, and this index is a major factor in controlling the turnover of crypt cells.

The relative rate of cell loss from the crypts of mice treated with DMH is greater than in control mice, but this increased rate of cell loss did not vary as a function of duration of DMH treatment. It follows that since the number of proliferative cells in the crypt gradually expanded as the number of DMH treatments increased and the relative rate of cell loss remained unchanged, the absolute number of cells lost from the crypts must increase to compensate for the increased birth rate of cells. Whether changes in the rates of cell loss from the crypts of DMH treated mice indicates the onset of neoplastic transformation is not known.

It has been postulated that retention of cells in either the upper (14) or lower (23) segments of the crypt may be a mechanism by which the normal balance of the crypt is disrupted by treatment with DMH and may underlie the formation of focal atypias and elongated crypts. In fact, the results from the present experiment do not support this, because retention of crypt cells in the upper crypt segments of DMH treated animals could not be demonstrated, and cells were lost at a faster rate than in control animals. However, these current results do not preclude two possible mechanisms involved in tumor formation in the colonic

mucosa: cells may be retained at the luminal surface for longer periods after treatment with carcinogens or an altered crypt cell may form a clone from which tumors arise. Lesions similar to these may have been present in the mucosa but went unrecognized because of the necessity of random selection of histologically-normal crypts for analysis of cell kinetics. A recent report that a single treatment with DMH causes tumors after 1.5 years (28) provides evidence that a single exposure may be sufficient to transform crypt cells and lends support to the theory of clonal origin of colon tumors. Multiple treatment with DMH may only serve to expose more cells to carcinogenic transformation and decrease the latency period of tumor formation (10,11,13,33).

In conclusion, the proliferative population of cells which expanded in response to DMH treatment may account for the increased rate of cell migration that leads to the premature appearance of labeled cells at the crypt surface and the increased rate of cell loss from the crypts. These changes in crypt dynamics may be an attempt to compensate for the perturbed kinetics induced by DMH treatment so that a constant lifespan for cells in the crypts is maintained. Whether these changes indicate development of neoplasia in the colonic epithelium is not clear, and it must be taken into consideration that random selection of viable crypts may or may not have included significant abnormalities.

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BIBLIOGRAPHY

1. Altmann, G.G. 1974 Changes in the Mucosa of the Small intestine Following Methotrexate Administration or Abdominal X-Irradiation. Am. J. Anat. 140: 263-280.

2. Barthold, S.W. 1979 Autoradiographic Cytokinetics of Colonic Mucosal Hyperplasia in Mice. 1979 Cancer Res. 39: 24-29.

3. Cairnie, A.G., L.F. Lamerton, G.G. Steel. 1965 Cell Proliferation Studies in the Intestinal Epithelium of the Rat. I. Determination of the Kinetic Parameters. Exp. Cell Res. 39: 528-538.

4. Cairnie, A.G., and R.E. Bentley. 1967 Cell Proliferation Studies in the Intestinal Epithelium of the Rat: Hyperplasia During Lactation. Exp. Cell Res. 46: 428-440.

5. Chang, W.W.L. 1978 Histogenesis of Symmetrical 1,2- Dimethyl-hydrazine Induced Neoplasms of the Colon in the Mouse. J. Natl. Cancer Inst. 60: 1405-1418.

6. Chang, W.W.L. 1981 Degenerative Behavior of Epithelial cells in the Colonic Crypt of the Mouse Following Administration of Colonic Carcinogen 1,2-Dimethylhydrazine. Cancer Lett. 13: 111-118.

7. Chang, W.W.L., and C.P. Leblond. 1971 Renewal of the Epithelium in the Descending Colon of the Mouse. I. Presence of Three Populations: Vacuolated-Columnar, Mucous and Argentaffin. Am. J. Anat. 131: 73-100.

8. Chang, W.W.L., and J.J. Nadler. 1975 Renewal of the Epithelium in the Descending Colon of the Mouse. IV. Cell Population Kinetics of Vacuolated-Columnar and Mucous Cells. Am. J. Anat. 144: 39-56.

9. Curtis, A.S.G., and T.E.J. Buultjens. 1973 Cell Adhesion and Locomotion. In Locomotion of Tissue Cells, Ciba Foundation Symposium 14. Associated Scientific Publishers: New York, NY, 171-194.

10. Deschner, E.E. 1974 Experimentally Induced Cancer of the Colon. Cancer Res. 34: 824-828.

11. Deschner, E.E., F.C. Long, A.P. Maskens. 1979 Relationship Between Dose, Time and Tumor Yield in Mouse Dimethylhydrazine-Induced Colon Tumorigenesis. Cancer Lett. 8: 23-28.

12. Hennings, H., and F. Devik. 1971 Comparison of Cytotoxicity of Hydroxyurea in Normal and Rapidly Proliferating Epidermis and Small Intestine in Mice. Cancer Res. 31: 277-282.

13. Izumi, K., H. Otsuka and K. Furuya. 1980 Carcinogenicity of 1,2-Dimethylhydrazine Dihydrochloride in Mice. Tokushima J. Exp. Med. 27: 5-13.

14. Lipkin, M. 1974 Phase 1 and Phase 2 Proliferative Lesions of Colonic Epithelial Cells in Diseases Leading to Colonic Cancer. Cancer 34: 878-888.

15. Lipkin, M., and H. Quastler. 1962 Cell Population Kinetics in the Colon of the Mouse. J. Clin. Invest. 41: 141-146.

16. Maskens, A.P. 1976 Histogenesis and Growth Pattern of 1,2-Dimethylhydrazine Induced Rat Colon Adenocarcinoma. Cancer Res. 36: 1585-1592.

17. Maurer, H.R. 1981 Potential Pitfalls of ^{3}H-Thymidine Techniques to Measure Cell Proliferation. Cell Tissue Kin. 14: 111-120.

18. Meyers, F.H., E. Jawetz and A. Goldfien. 1978 Review of Medical Pharmacology, 6th Edition. Lange Medical Publications p. 488.

19. Messier, B., and C.P. Leblond. 1960 Cell Proliferation and Migration as Revealed by Radioautography after Injection of ^{3}H-Thymidine into Male Rats and Mice. Am. J. Anat. 106: 247-285.

20. Potten, C.S., and L. Kovacs. 1973 An Estimation of Proliferative Population Size in Stomach, Jejunum and Colon of DBA-2 Mice. Cell Tissue Kin. 6: 125-134.

21. Quastler, H., and F.G. Sherman. 1959 Cell Population Kinetics of the Intestinal Epithelium of the Mouse. Exp. Cell Res. 17:420-438.

22. Richards, T.C. 1977 Early Changes in the Dynamics of Crypt Cell Populations in Mouse Colon following Administration of 1,2-Dimethylhydrazine. Cancer Res. 37: 1680-1685.

23. Richards, T.C. 1981 The Effects of the Carcinogen, 1,2,-Dimethylhydrazine, on Turnover of ^{3}H-Thymidine Labeled Cells from Mucosal Glands of Mouse Colon. Anat. Rec. 200: 299-308.

24. Richards, T.C. 1981 Changes in Crypt Cell Populations of Mouse Colon During Recovery from Treatment with 1,2-Dimethylhydrazine. J. Natl. Cancer Inst. 66: 907-912.

25. Rijke, R.P.C., W. van der Meer-Fieggen and H. Galjaard. Effects of Villus Length of Cell Migration in Small Intestinal Epithelium. Cell Tissue Kin. 7: 577-586.

26. Rogers, A.W. 1979 Techniques of Autoradiography, 3rd Edition. Elsevier/North-Holland Biomedical Press.

27. Sawicki, W., J. Rowinski, W. Maciejewski and K. Kwarecki. 1968 Kinetics of Proliferation and Migration of Epithelial Cells in the Guinea Pig Colon. Exp. Cell Res. 50: 93-103.

28. Schiller, C.M., W.H. Curley and E.E. McConnell. 1980 Induction of Colon Tumors by a Single Oral Dose of 1,2-Dimethylhydrazine. Cancer Lett. 11: 75-79

29. Sherman, F.G., and H. Quastler. 1960 DNA Synthesis in Irradiated Intestinal Epithelium. Exp. Cell Res. 19: 343-360.

30. Snedecor, G.W., and W.G. Cochran. 1980 Statistical Methods, 7th Edition. The Iowa State University Press. P. 325-329.

31. Sunter, J.P., D.R. Appleton and A.J. Watson. 1981 Acute Changes Occurring in the Intestinal Mucosae of Rats Given a Single Injection of 1,2-Dimethylhydrazine. Virchows Arch. B. Cell Path. 36: 47-57.

32. Tacha, D.E., and T.C. Richards. 1981 Comparison of Glycol Methacrylate- and Paraffin-Embedded Tissues for Identification for Tritium-Labeled Cells After Emulsion Radioautography. J. Histotech. 4: 59-62.

33. Thurnherr, N., E.E. Deschner, E.H. Stonehill, and M. Lipkin. 1973 Induction of Adenocarcinomas of the Colon in Mice by Weekly Injections of 1,2-Dimethylhydrazine. Cancer Res. 33: 940-945.

34. Tsubouchi, S. 1981 Kinetic Analysis of Epithelial Cell Migration in the Mouse Descending Colon. Am. J. Anat. 161: 239-246.

35. Tutton, P.J.M., and D.H. Barkla. 1978 Cell Proliferation in Dimethylhydrazine-Induced Colonic Adenocarcinomata Following Cytotoxic Drug Treatment. Virchows Arch. B. Cell Path. 28: 151-156.

36. Young, C.W., and S. Hodas. 1964 Hydroxyurea; Inhibitory Effect on DNA Metabolism. Science 146: 1172-1174.

37. Young, C.W., G. Schochetman, and D.A. Karnofsky. 1967 Hydroxyurea-Induced Inhibition of Deoxyribonucleotide Synthesis: Studies in Intact Cells. Cancer Res. 27: 526-534.

Figure Ia EXPERIMENTAL PROTOCOL I (N=252)

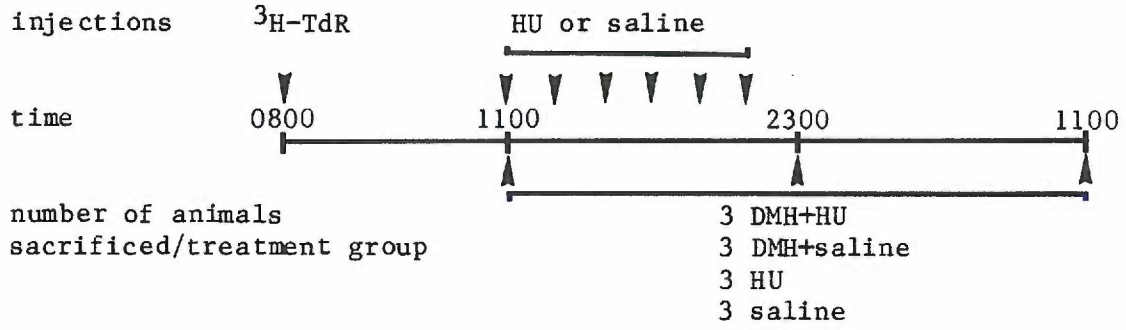
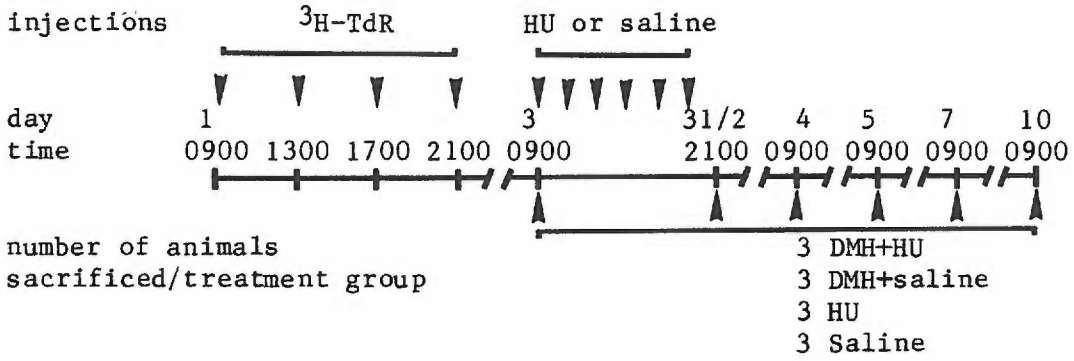


Figure 1b EXPERIMENTAL PROTOCOL II (N=432)



One week following termination of DMH treatments for 1,2,4,8,12,16 and 20 weeks, DMH and control animals were subjected to the appropriate protocol above. ^{3}H-TdR=^{3}H-thymidine; HU=hydroxyurea; DMH+HU=animals treated with DMH and hydroxyurea; DMH+saline=animals treated with DMH and saline; HU=animals treated with hydroxyurea only; Saline=untreated control animals. Three animals per subgroup were sacrificed at each indicated time interval.

Figure II. Number of cells in the proliferative and non-proliferative zones of colon crypts during DMH treatment. Shaded and unshaded bars are mean values with S.E.M. indicated.

* significantly different from control values ($p<.05$)

** significantly different from control values ($p<.01$)

FIGURE 11

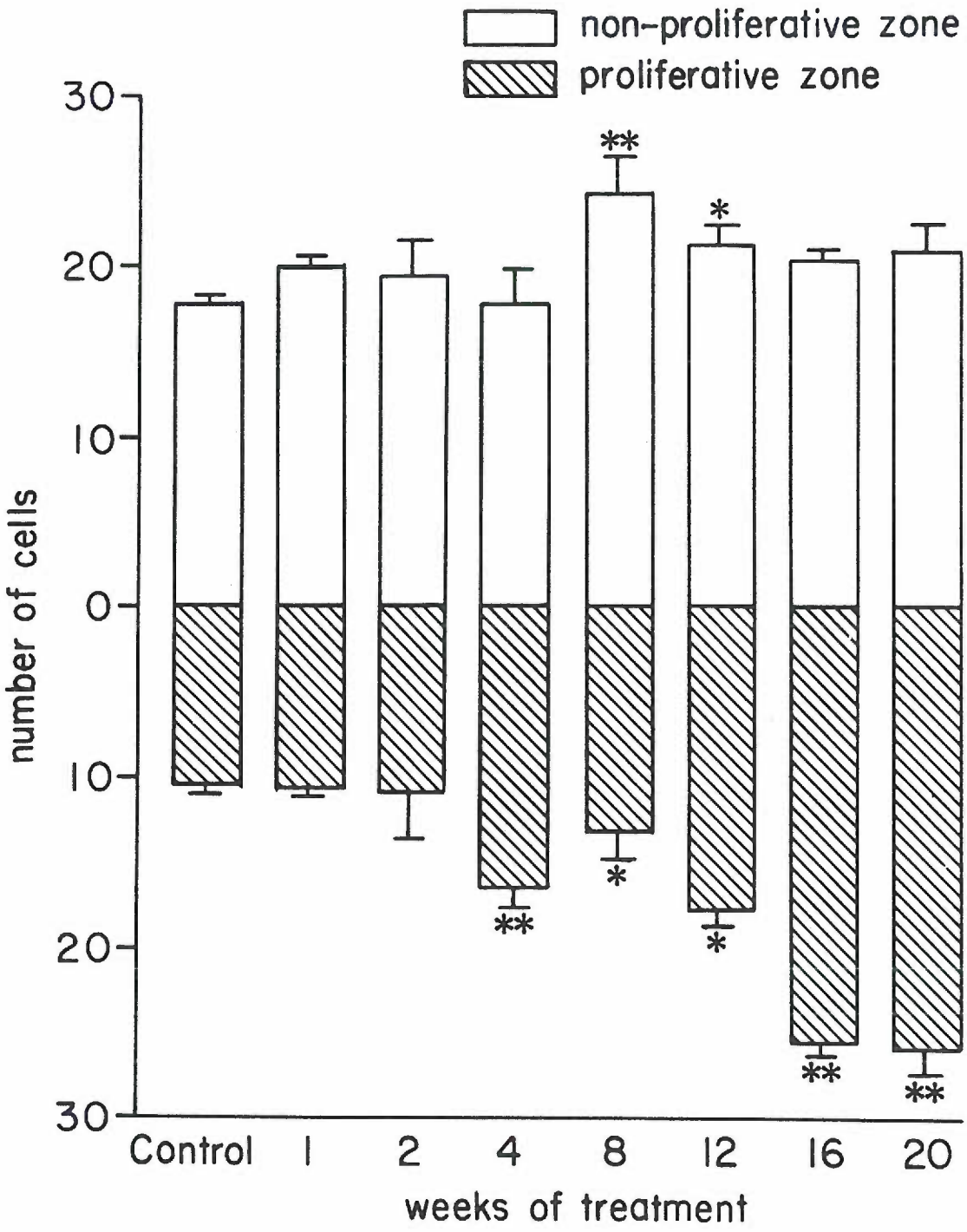

non-proliferative zone
proliferative zone
30
20
10
0
10
20
30
number of cells
**
*
*
**
*
**
**
Control
1
2
4
8
12
16
20
weeks of treatment

Figure III. Relative rates of cell loss from the colon crypts of control and DMH treated mice. The loss of cells from the crypts is represented by the decrease in LI. Each point represents the mean of values for a treatment week. Relative rates of cell loss in the DMH and control groups were significantly different ($p<.01$) when tested with nested analysis of variance.

FIGURE III

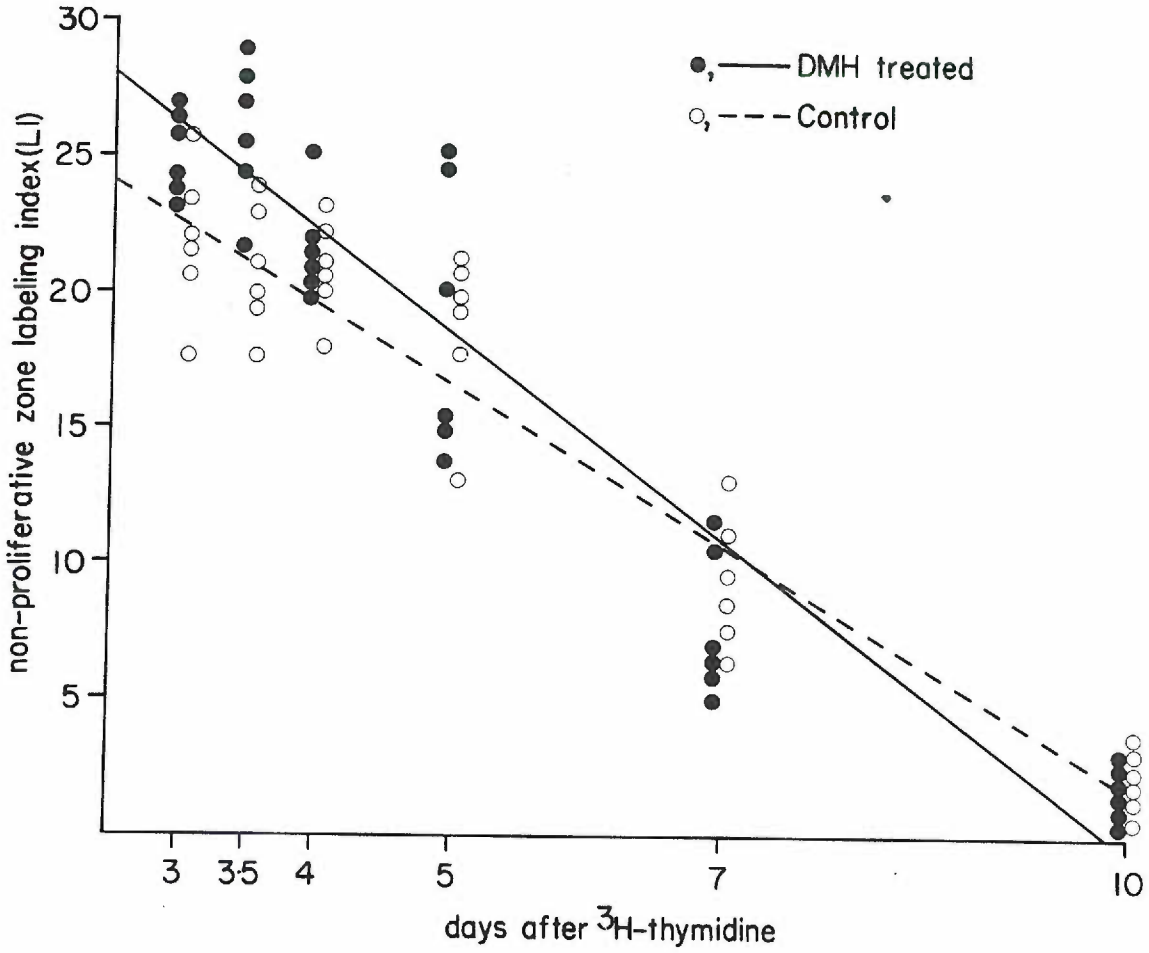

30
25
20
15
10
5
non-proliferative zone labeling index(LI)
●, —— DMH treated
○, - - - Control
3
3·5
4
5
7
10
days after ^{3}H-thymidine

Figure IV. Autoradiographs of colon crypts in control mice. Plate a, 3 hours after ^{3}H-thymidine. Plate b, 27 hours after ^{3}H-thymidine. Arrow depicts position of highest labeled cell. 160X

Autoradiographs of hyperplastic colon crypts in mice treated with DMH for 20 weeks. Plate c, 3 hours after ^{3}H-thymidine. Plate d, 27 hours after ^{3}H-thymidine. Arrow depicts position of highest labeled cell. 160X

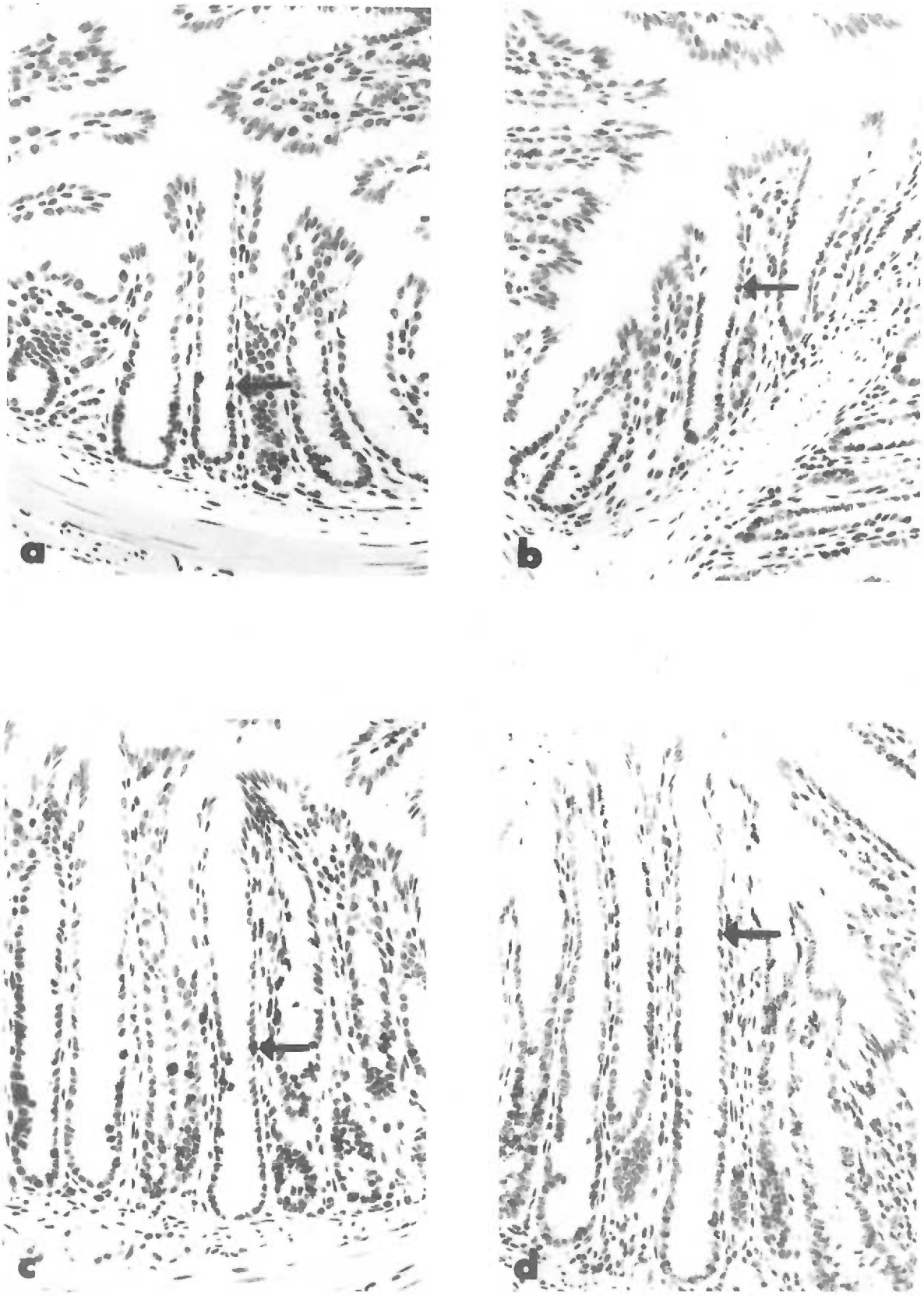

a
b
c
d

TABLE I

CHANGES IN CRYPT LENGTH, LABELING INDEX AND RATE OF CRYPT CELL MIGRATION DURING DMH TREATMENT

TREATMENTS	CRYPT LENGTH (no. of cells)	LABELING INDEX(LI)[a]	RATE OF CRYPT CELL MIGRATION (cell positions per hour)
Control (63)[b]	28.8±0.3[c]	13.5±0.9	0.257±0.02
1wk DMH (9)	31.6±0.6**	12.6±0.6	0.284±0.08
2wk DMH (9)	31.3±0.5**	13.1±2.7	0.239±0.06
4wk DMH (9)	34.4±0.6***	20.5±2.0**	0.158±0.16
8wk DMH (9)	37.3±0.5***	14.8±2.1	0.414±0.17*
12wk DMH (9)	39.0±0.7***	15.4±1.2	0.336±0.07*
16wk DMH (9)	45.7±0.6***	26.8±0.5**	0.336±0.01*
20wk DMH (9)	46.5±1.4***	17.3±2.5*	0.524±0.03**

[a] $LI = \frac{\text{total labeled cells/crypt}}{\text{total cells/crypt}} \times 100$

[b] number of animals in parentheses

[c] Mean ± S.E.M.

* significantly different from control values ($p<.05$)

** significantly different from control values ($p<.01$)

*** significantly different from control values ($p<.001$)

TABLE II

EFFECTS OF HYDROXYUREA ON THE RATE
OF CELL MIGRATION IN COLON CRYPTS
(cell positions per hour)

HOURS[a]	CONTROL	HU ONLY	DMH ONLY[a]	DMH+HU[c]
1100-2300	0.276±0.05	-0.046±0.05***	0.393±0.05*	0.075±0.07**
2300-1100	0.237±0.06	0.184±0.01*	0.296±0.06*	0.045±0.09*

[a]Pulse label of ^{3}H-thymidine was given at 0800 hours of day 1. Rates of cell migration were determined from the change in position of the leading edge of labeled cells in animals sacrificed at 1100, 2300 (day 1) and 1100 hours (day 2).

[b]Mean values for DMH only treatment weeks

[c]Mean values for DMH+HU treatment weeks

* significantly different from control values ($p<.05$)
** significantly different from control values ($p<.01$)
*** significantly different from control values ($p<.001$)

TABLE III

LABELING INDEX OF THE CRYPT NON-PROLIFERATIVE ZONE
IN CONTROL AND DMH-TREATED MICE

	NON-PROLIFERATIVE ZONE LABELING INDEX	
DAYS AFTER ^{3}H-THYMIDINE	DMH TREATMENT[a]	CONTROL[b]
3	25.0±0.7**	21.8±1.1
3 1/2	26.3±1.2**	20.9±0.9
4	21.6±0.7	20.9±0.7
5	19.0±2.1	18.7±1.2
7	7.8±1.1	9.3±1.0
10	1.4±0.3	1.8±0.4

[a]All DMH treatment weeks combined; see text
[b]All Control treatment weeks combined; see text
** significantly different from control values ($p<.01$)

IV. Appendix

INTRODUCTION

Cells of the mitotically active population in the base of the colon crypts migrate upward toward the luminal surface where they are sloughed into the lumen of the colon. The system is normally in a steady state balanced by the birth of new cells and the loss of senescent cells (4,5). Although the mechanisms that control migration of cells are not well understood, one factor that may contribute to this process is the pressure exerted by dividing cells in the crypt base.

The effects of inhibition of mitosis on the migration of ^{3}H-thymidine labeled crypt cells can be determined by blocking mitosis with vinblastine sulfate. Vinblastine inhibits the polymerization of synthesizing microtubular proteins and blocks cells in mitosis at metaphase. If the migration of cells is independent of mitotic activity, the leading edge of labeled crypt cells should be the same as that of controls after mitotic inhibition. Alternatively, if crypt cell migration is dependent upon the division of cells in the base of crypt, the leading edge of labeled cells should trail those of the control group following treatment with vinblastine. Vinblastine has also been reported to disrupt existing microtubules associated with the cell membrane and disturb the regulation of DNA synthesis (9,10). Therefore,

vinblastine apparently alters processes that may also contribute to cell migration and is not a viable drug to determine those events that are essential to movement of cells in crypts. Although the results do not necessarily distinguish between the roles of cell division and microtubule synthesis in cell migration, these and other observations are presented here.

Preliminary experiments were performed to determine: (1) the rate of accumulation of mitotic figures following single and multiple injections of vinblastine, (2) the physiologic half-life of vinblastine as evidenced by the accumulation of mitoses, (3) the histologic confirmation of accumulated cells arrested at metaphase and (4) the validity of radioautography as an accurate method to document the migration of labeled crypt cells.

MATERIALS AND METHODS

I. Determination of a Mitotic Accumulation Curve

Thirty-one female Swiss Webster mice (20-25 grams BW) were housed 5 animals per cage with free access to food and water in animal quarters (22°C) with a 12 hour light/dark cycle. All animals were injected intraperitoneally (i.p.) with vinblastine sulfate (VB; Eli Lilly and Co.; Indianapolis, IN) at 1.0 mg/kg body wt. every 3 hours for 12 hours. Beginning with the initial injection of VB, a single animal was sacrificed at 15 minute intervals for 3 hours; thereafter animals were sacrificed at 30 minute intervals up to 12 hours. Tissue from the descending colon at the level of the pelvic rim was prepared for histologic examiantion and crypts were selected for analysis as previously described. The total number of cells and the number of mitotic figures in 20 crypt columns were recorded. To determine the mitotic index (MI), the number of mitoses per crypt was divided by the number of cells per crypt column times 100. A mean MI for each animal was obtained from the values of 20 crypt columns. A mitotic accumulation curve was constructed by plotting the mean MI for each animal over time of treatment with VB, and the data were treated by linear regression analysis.

II. Radioautographic Analysis of Cell Migration after Mitotic Inhibition

Thirty-six female Swiss Webster mice (20-25 grams BW) were cared for as described above. All animals were given a pulse label of ^{3}H-thymidine i.p. (0.5 Ci/gram body wt.; specific activity, 20 Ci/mmole, (New England Nuclear; Boston, MA) at 0800 hours. One hour later the animals were divided into two groups and injected with either VB (1.0 mg/kg BW) or equal volumes of normal saline every 3 hours for 12 hours. Animals were sacrificed in groups of 3 at the following times after ^{3}H-thymidine: 1 hour (at the beginning of VB treatment), 12 hours (at the end VB treatment), 2,3,4 and 5 days. Sections of colon were prepared for radioautography as described previously. Forty crypt columns per animal were analyzed for the total number of cells and the number and position of labeled cells. The mean labeling index (LI) for each animal was calculated from the percentage of labeled cells per crypt column. Cell migration (cell positions per hour) was determined as described previously. The significance of differences in crypt size, LI and the migration of the leading edge of labeled cells in the VB treated and control groups was determined with Student's t test.

RESULTS

I. Accumulation of Mitotic Figures

There was a progressive increase in the number of crypt cells arrested at metaphase over time (Figure I). At 0 and 15 minutes after the initial injection of VB, the mitotic index (MI) was the same as control values. However, by 30 minutes the MI was elevated above control values ($p<.05$) and by 12 hours the MI had increased to a level of 20% ($p<.001$).

II. Crypt Cell Migration After Vinblastine

Cell migration was inhibited during treatment with VB (Table I). After termination of treatment with VB crypt cell migration resumed at a rate that was not significantly different from the control group until day 5, when the rate of cell migration increased in response to a regenerative surge of cells in the crypts.

Changes in the crypt population observed during the experiment are given in Table II. Crypt length in animals treated with VB was not different from the control groups for up to 4 days after the termination of treatment but increased to 42.8 cells on day 5 ($p<.01$). All groups treated with VB for 12 hours had a LI that was less than comparable control values ($p<.05$).

DISCUSSION

The rate of accumulation of arrested mitoses in the present study was less than previous reports (6,11). This difference may have been due to the variability in the animals or the choice of vinblastine as the mitotic inhibitor which is less effective than vincristine (12). The results demonstrate the inhibition of cell migration along the crypt column occurred only during the treatment with VB and that migration of cells resumed at normal rates after VB treatment was terminated. This suggests that the effects of VB are of rather short duration (2,3), and that cells rapidly recover from these effects. Although migration of crypt cells was inhibited during treatment with VB, the lack of cell migration cannot be entirely attributed to blockage of cell division. Since VB also disrupts microtubules associated with the cell membrane, the contribution of microtubule formation believed to be involved in amoeboid migration of cells can not be excluded (9). A similar experimental approach using cytotoxic agents that inhibit the division of cells but do not disrupt microtubule formation would be valuable for determining the role of cell division in crypt cell migration. Hennings and Devik (8) observed that labeled cells did not migrate as far up the small intestinal villi as in controls after treatment with hydroxyurea suggesting that disruption of proliferative activity in the crypts slowed the migration of villous cells. Since hydroxyurea inhibits an

enzyme pathway involved in DNA synthesis prior to mitosis and does not affect the cellular ultrastructure of microtubules as does VB, it may be a more suitable agent for determining the role of mitosis in cell migration in the intestinal crypts.

The rebound in size of the crypt length that occurred 5 days after VB treatment may be in response to disruption of microtubules which are considered to be important in the regulation of DNA synthesis (9). Although Tutton and Barkla (13) described a more immediate acceleration of cell proliferation during the period of recovery from 5-fluorouracil, the recovery from VB treatment in the present experiment occurred several days later. This difference in recovery may also reflect the degree of disruption caused in the crypt cell population. Crypt and villous populations in the small intestine have a similar hyperproliferative response after irradiation (7). The rapid increase in cell migration to 0.68 cells/hour from day 4 to day 5 may be explained by the rapid proliferation of cells in the base of the crypt that resulted in a large apparent change in the position of the highest labeled cells. This response is similar to the increased rate of crypt cell migration that occurs in mice with bacterially induced crypt hyperplasia (1).

The results do not demonstrate conclusively that migration of crypt cells in the colon is dependent upon cells dividing in the base of the crypt, but suggest that the rate of crypt cell migration may be related to cell prolifertion in the crypt population.

BIBLIOGRAPHY

1. Barthold, S.W. 1979 Autoradiographic Cytokinetics of Colonic Mucosal Hyperplasia in Mice. Cancer Res. 39: 24-29.

2. Beer, C.T., M.L. Wilson and J. Bell. 1964 The Metabolism of Vinca Alkaloids. Part I. Can. J. Physiol. Pharmacol. 42: 1-11.

3. Beer, C.T. and J.F. Richards. 1964 The Metabolism of Vinca Alkaloids. Part II. Lloydia 27: 352-360.

4. Chang, W.W.L. and C.P. Leblond. 1971 Renewal of the Epithelium in the Descending Colon of the Mouse. I. Presence of Three Cell Populations: Vacuolated-Columnar, Mucous and Argentaffin. Am. J. Anat. 131: 73-100.

5. Chang, W.W.L. and N.J. Nadler. 1975 Renewal of the Epithelium in the Descending Colon of the Mouse. IV. Cell Population Kinetics of Vacuolated-Columnar and Mucous Cells. Am.J. Anat. 144: 39-56.

6. de Rodriguez, M.S.B., J.P. Sunter, A.J. Watson, N.A. Wright and D.R. Appleton. 1979 Cell Population Kinetics in the Mucosal Crypts of the Descending Colon of the Mouse. Virchows Arch. B. Cell Path. 29: 351-361.

7. Hagemann, R.F. and S. Lesher. 1971 Irradiation of the G.I. tract: compensatory response of stomach, jejunum and colon. Brit. J. Radiol. 44: 599-602.

8. Hennings, H. and F. Devik. 1971 Comparison of Cytotoxicity of Hydroxyurea in Normal and Rapidly Proliferating Epidermis and Small Intestine in Mice. Cancer Res. 31: 277-282.

9. Otto, A.M. 1982 Microtubules and the Regulation of DNA Synthesis in Fibroblastic Cells. Cell Biol. Int. Rep. 6: 1-18.

10. Sloboda, R.D. 1980 The Role of Microtubules in Cell Structure and Cell Division. Am. Sci. 68: 290-298.

11. Sunter, J.P., N.A. Wright and D.R. Appleton. 1978 Cell Population Kinetics in the Epithelium of the Colon of the Male Rat. Virchows Arch. B. Cell Path. 26: 275-287.

12. Tannock, I.F. 1965 A Comparison of the Relative Efficiencies of Various Metaphase Arrest Agents. Exp. Cell Res. 47: 345-356.

13. Tutton, P.J.M. and D.H. Barkla. 1978 Cell Proliferation in Dimethylhydrazine-Induced Colonic Adenocarcinomata Following Cytotoxic Drug Treatment. Virchows Arch. B. Cell Path. 28: 151-156.

Figure I. Accumulation of Mitotic Figures after Vinblastine. Vinblastine was given at 0,3,6 and 9 hours as indicated by the arrows. MI=[(number of mitotic cells/total number of cells) x 100]. Each point represents the mean MI of 20 crypt columns for a single animal. Data was treated with regression analysis ($r=+0.90$, $y=4.25+1.08x$, $N=31$).

FIGURE 1

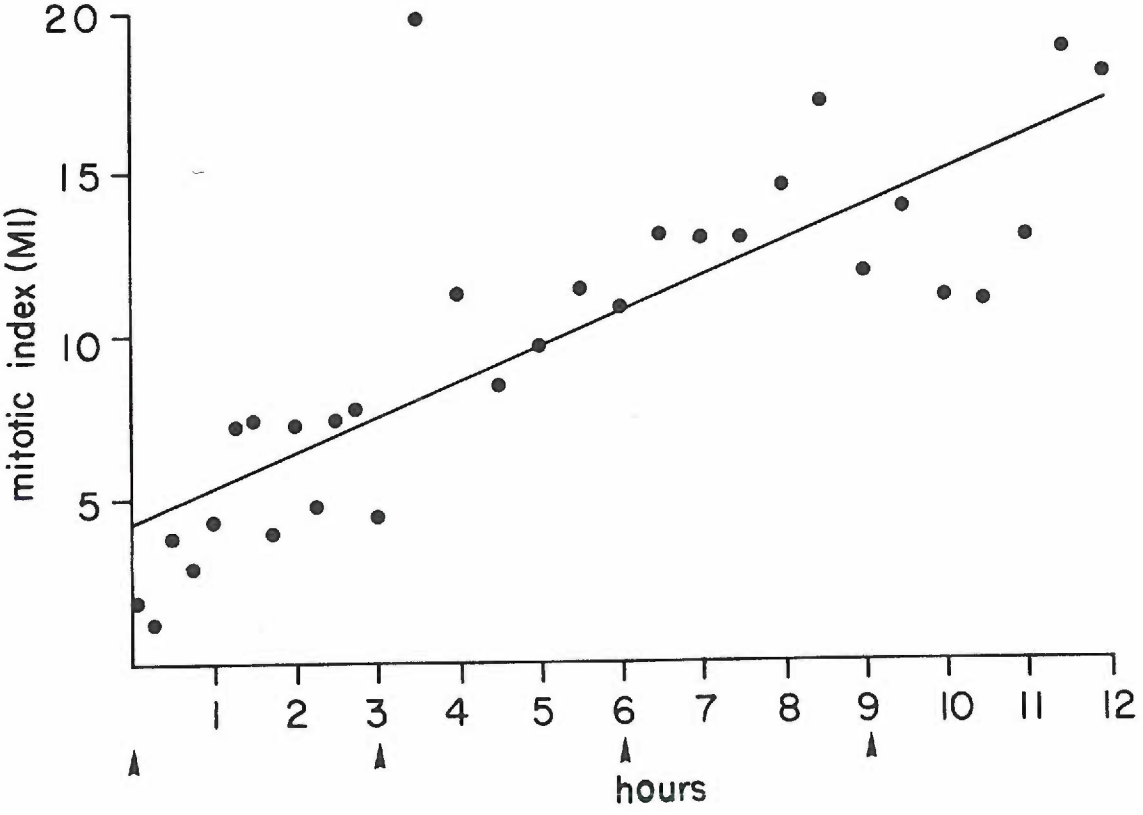


TABLE I

EFFECTS OF VINBLASTINE ON THE RATE OF CELL MIGRATION IN COLON CRYPTS
(cell positions per hour)

TIME AFTER ^{3}H-THYMIDINE	VINBLASTINE TREATED	CONTROL
12 hours	0.10±0.02[a]*	0.24±0.01
24 hours (day 2)	0.26±0.08	0.28±0.03
day 3	0.23±0.06	0.21±0.08
day 4	0.20±0.16	0.20±0.11
day 5	0.68±0.17*	0.18±0.13

[a]Mean±S.E.M.

* significantly different from control values ($p<.05$)

TABLE II

CHANGES IN CRYPT LENGTH AND LABELING INDEX
IN VINBLASTINE TREATED AND CONTROL ANIMALS

TIME AFTER ^{3}H-THYMIDINE	VINBLASTINE TREATED		CONTROL	
	CRYPT LENGTH[a]	LI[b]	CRYPT LENGTH	LI
1 hour	31.1±0.6[c]	13.0±1.9	31.1±0.6	13.0±1.9
12 hours	29.2±0.3	11.3±1.4*	31.0±0.4	17.8±1.0
2 days	28.4±0.5	15.9±1.9*	29.4±0.9	24.5±4.9
3 days	30.0±1.2	14.0±1.4*	29.0±0.8	22.8±1.4
4 days	33.6±3.3	11.9±3.7*	30.0±0.3	19.7±0.3
5 days	42.8±3.0**	11.5±2.1*	29.5±0.8	21.3±1.3

[a]number of cells per crypt column

[b]$LI = \frac{\text{total labeled cells/crypt}}{\text{total cells/crypt}} \times 100$

[c]Mean±S.E.M.

* significantly different from control values ($p<.05$)
**significantly different from control values ($p<.01$)

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