[CANCER RESEARCH 36; 13–17, January 1976]

Early Effects of a Single Intrarectal Dose of 1,2-Dimethylhydrazine in Mice[1]

P. C. Chan, L. A. Cohen, T. Narisawa, and J. H. Weisburger

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595

SUMMARY

The early morphological and biochemical effects of intrarectally administered 1,2-dimethylhydrazine dihydrochloride on mouse colon were studied. Using [^{3}H]thymidine autoradiography, it was found that 1,2-dimethylhydrazine dihydrochloride, 250 mg/kg decreased the number of prelabeled DNA-synthesizing cells in the distal colon as early as 30 min after instillation. During the interval from 24 hr to 2 weeks, however, the opposite effect was seen; incorporation of [^{3}H]thymidine increased 3- to 5-fold over controls. At lower doses (0.25 to 25 mg/kg), a similar trend was observed. Histological examination showed no dramatic changes in cell structure or in tissue architecture. No changes were seen in labeling indices in the proximal colon. In the liver, cellular alterations were seen at concentrations of 25 to 250 mg/kg, particularly in the centrolobular region. These changes were evident at 2 hr and disappeared by 4 hr. The kidney was unaffected by 1,2-dimethylhydrazine dihydrochloride at any concentration.

Our results suggest that enzymes capable of activating 1,2-dimethylhydrazine dihydrochloride are located within the mucosal cells of the distal colon.

INTRODUCTION

The symmetrical compound, DMH,[2] is 1 of the best chemicals known to reliably induce colon adenocarcinomas in rodents (4). The DMH tumor closely resembles human colon cancer in morphology and development and therefore provides a good model for the understanding of human colon cancer (8, 9, 16, 17).

Although it is generally agreed that DMH is not a direct-acting carcinogen, the precise mode of activation requires clarification. Metabolism of DMH to an alkylating carcinogen could conceivably involve liver, bacterial flora, mucosal enzymes, or any of these in combination (10, 22).

One approach to the understanding of the mode of action of DMH is by the study of the early changes that occur in colon mucosal cells following administration of the compound. Löhrs *et al.* (9) reported that a single s.c. dose of DMH induced specific cytological damage to the colonic mucosa, a disturbance in crypt cell proliferation kinetics, and a marked inhibition of the migration of differentiated cells along the crypt as early as 8 hr postadministration.

In the present report we also deal with the early changes induced by DMH. Our study was carried out in the light of 2 facts not previously considered. Since carcinogens may act on cells practically instantaneously (3), we began observations within 30 min of DMH administration. Since the tumor-producing potency of DMH is reduced in germ-free rats compared to conventional rats (17, 18), we used the method of i.r. instillation (11), a technique by which the interaction of DMH with the colonic flora and mucosal cells is immediate and the direct influence of the liver is minimized.

MATERIALS AND METHODS

DMH (M. W. 133; Schuchardt, Munich, Germany) was dissolved in PBS (Grand Island Biological, Grand Island, N.Y.) and adjusted to pH 6.5 with NaOH. The solution was used immediately in order to prevent decomposition (14).

Female Swiss (ICR/Ha) mice (144, 11 weeks old) from ARS/Sprague-Dawley, Madison, Wis., were divided into 6 groups (24 mice in each group). Food (Purina laboratory chow) was withheld for 24 hr. Animals in Groups 1 to 4 received a single i.r. dose of DMH at 0.25 mg/kg (1.88 μmoles/kg), 2.5 mg/kg (18.8 μmoles/kg), 25 mg/kg (188 μmoles/kg), and 250 mg/kg (1.88 mmoles/kg), respectively, with a bulbous-tipped needle in 0.05 ml PBS. Group 5 received the vehicle only; Group 6 was not treated and served as controls. The technique of administering carcinogens and test chemicals i.r. has been used repeatedly in our laboratory for colon tumor induction in mice (12), rats (11), and guinea pigs (13). Preliminary studies indicated that 100% of the mice were killed 3 days after an i.r. injection of DMH, 500 mg/kg, whereas all the mice survived a dose of 250 mg/kg.

To evaluate the effect of DMH on prelabeled DNA-synthesizing cells, 3 mice from each group were given an i.p. injection of 30 μCi [^{3}H]thymidine (specific activity, 17.9 Ci/mmole; Schwarz/Mann, Orangeburg, N.Y.) 30 min before, and sacrificed at 30 min, 2 hr, and 4 hr after, i.r. DMH or vehicle instillation.

The proliferative rate (labeling index) of crypt cells after DMH treatment was studied by giving an i.p. injection of 30 μCi [^{3}H]thymidine to 3 mice in each group 1 hr before sacrifice 1, 2, 3, 7, and 14 days after DMH or vehicle instillation.

[1] This investigation was supported by USPHS Research Grants CA-12376 from the National Cancer Institute and CA-15400 through the National Large Bowel Cancer Project.

[2] The abbreviations used are: DMH, 1,2-dimethylhydrazine dihydrochloride; i.r., intrarectal; PBS, phosphate-buffered saline (components/liter: 100 mg $CaCl_2$, 200 mg KCl, 200 mg KH_2PO_4, 100 mg $MgCl_2 \cdot 6H_2O$, 8000 mg NaCl, and 150 mg $Na_2HPO_4 \cdot 2H_2O$).

Received March 7, 1975; accepted September 10, 1975.

At autopsy, a 2-cm segment of the colon about 1 cm from the anus (distal colon) and a similar segment, serving as a control not bathed directly by the carcinogen, adjacent to the cecum (proximal colon) were removed, fixed in neutral formalin, embedded in paraffin, and sectioned transversely at 3 to 5 μm thickness. Fixed sections were then dipped in Kodak NTB2 photographic emulsion, stored at 4° for 3 weeks, and developed for autoradiographic examination (2). The labeling index (labeled nuclei in crypt per total nuclei in crypt) × 100% was estimated by counting cells in 30 or more crypts in each section. Only cells from crypts displaying an entire longitudinal section were counted. Background grains were negligible; individual cells having 4 or more grains over the nuclei were considered labeled (Fig. 1). Differences in labeling index between the DMH-treated and control groups were evaluated statistically by the Student's *t* test. Labeling indices for combined vehicle and untreated controls were calculated by pooling the labeling indices of all 24 mice in Groups 5 and 6, respectively.

Acridine orange (Sigma Chemical Co., St. Louis, Mo.) staining (20) was used to distinguish histochemically between mucus-containing cells and active dividing cells in the crypts. The sections were studied with an Olympus fluorescent microscope, using excitation filter B_2 and barrier filter OG515. Under these conditions, cells in the lower portion of the crypt stained bright yellow-red, whereas mucopolysaccharide-containing differentiated cells in the upper crypt appeared grayish-green.

Sections of liver and kidney obtained from each mouse were also prepared and stained with hematoxylin and eosin. For liver, the periodic acid-Schiff stain for glycogen was performed as described by Thompson and Hunt (20).

RESULTS

Distal Colon. The effect of DMH on prelabeled DNA-synthesizing cells in the lower one-third of the crypt in the distal colon is presented in Table 1. Compared to the combined vehicle or untreated controls, a dose of DMH, 0.25 mg/kg, had no apparent effect on the number of prelabeled cells within 2 hr. By 4 hr the number of labeled cells was lowered. At a dose of 2.5 to 250 mg/kg, DMH significantly decreased the prelabeled cells 30 min to 4 hr afterward, except in the group 30 min after receiving DMH, 25 mg/kg. Interestingly, routine light microscopic examination of stained sections of the entire crypt of the distal colon did not reveal any overt cytotoxic effect within 4 hr after DMH instillation.

From 24 hr to 2 weeks afterward, a dose of DMH, 250 mg/kg, induced a significant increase in labeling indices in the distal colon as compared to the values of the overall controls (Table 2). At lower doses (0.25 to 25 mg/kg), DMH caused a significant elevation in proliferative rate, except in Group 1, Day 7; Group 2, Days 2 and 14; and Group 3, Day 2. In the 2-week period of observation after DMH instillation, there was no increased migration of DNA-synthesizing cells up the crypt. [^{3}H]Thymidine incorporation was confined mainly to cells located at the bottom one-third of the crypt.

It can be seen in Tables 1 and 2 that the technique of i.r. instillation itself did not cause any significant change in labeling indices, since the combined values in vehicle controls were not different from those in the untreated controls.

Routine histological examination did not reveal any abnormal morphology, differences in crypt height, or any degenerating or pyknotic cells in the crypt when compared to control cells, except for the appearance of inflammatory

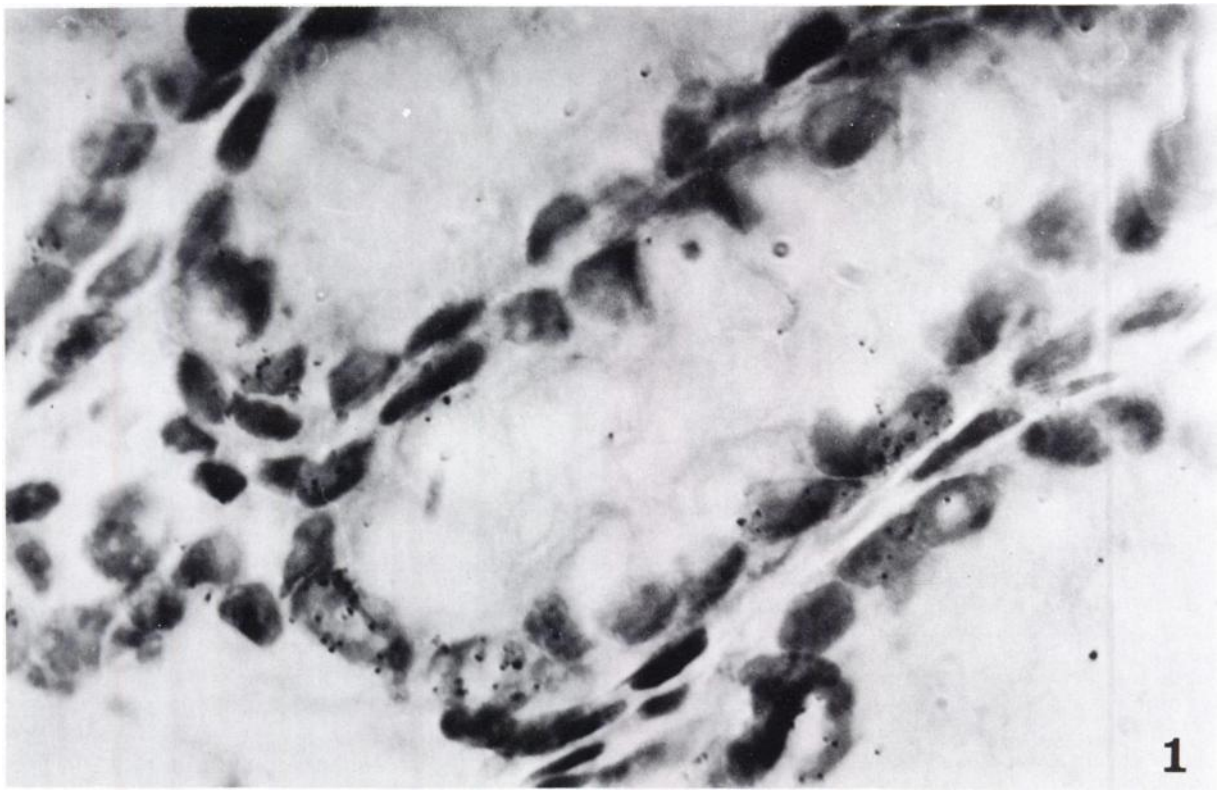

Fig. 1. Microautoradiograph of lower one-third of distal colon mucosa. The majority of grains are located over individual cell nuclei. × 1342.

Table 1

Effects of a single i.r. instillation of DMH on prelabeled DNA-synthesizing cells in the distal colon

[^{3}H]Thymidine was injected i.p. 30 min before, and the animals were sacrificed at 0.5, 2, and 4 hr after, DMH or vehicle instillation. Crypts in longitudinal section were measured and divided into 3 equal segments. The labeling indices in the lower one-third of the crypt are presented. In the upper and middle one-third of the crypts, no labeling was observed.

Group	Dosage (mg/kg)	Labeling indices		
		0.5 hr	2 hr	4 hr
1	0.25	6.5 ± 1.4[a]	5.6 ± 2.0	2.9 ± 0.5[b]
2	2.5	1.4 ± 1.0[b, c]	1.7 ± 0.9[b, c]	2.4 ± 0.6[b]
3	25	4.5 ± 0.8	1.9 ± 0.2[b, c]	2.6 ± 1.0[b]
4	250	1.8 ± 0.8[b, c]	1.2 ± 0.5[c, d]	1.1 ± 0.3[c, d]
5	Vehicle control[e]	6.8 ± 2.0	4.2 ± 0.5	5.2 ± 1.1
6	Untreated control[f]	4.2 ± 1.0	2.9 ± 0.5	3.7 ± 0.7

[a] Mean ± S.E.
[b] $p \leq 0.05$ *versus* combined vehicle control.
[c] $p \leq 0.05$ *versus* combined untreated control.
[d] $p \leq 0.01$ *versus* combined vehicle control.
[e] Combined vehicle control (mean labeling index of 24 mice), 5.7 ± 0.4.
[f] Combined untreated control (mean labeling index of 24 mice), 4.8 ± 0.4.

Table 2

Effects of a single i.r. instillation of DMH on cell proliferation in the distal colon

[^{3}H]Thymidine was injected i.p. 1 hour before the animals were sacrificed at 24 hr, 48 hr, 72 hr, 1 week, and 2 weeks after DMH or vehicle instillation. Here, labeling indices in the lower one-third of crypts in longitudinal sections are presented. No labeling was observed in the upper and middle one-third of the crypts.

Group	Dosage (mg/kg)	Labeling indices				
		1 day	2 days	3 days	7 days	14 days
1	0.25		9.5 ± 2.3[a, b]	9.2 ± 0.7[b, c]	6.7 ± 1.0	10.8 ± 3.5[b, c]
2	2.5	8.1 ± 1.0[a, b]	6.8 ± 1.0	7.8 ± 1.0[d]	11.3 ± 2.2[c, e]	7.6 ± 1.8
3	25	11.8 ± 0.7[c, e]	7.6 ± 1.4	18.6 ± 2.8[c, e]	8.5 ± 2.5[b, d]	17.0 ± 6.8[c, e]
4	250	12.7 ± 4.3[e]	16.8 ± 0.4[c, e]	12.5 ± 1.0[c, e]	10.3 ± 1.7[b]	16.4 ± 2.5[c, e]
5	Vehicle control[f]	6.6 ± 0.8	5.1 ± 1.3	3.8 ± 1.3	8.8 ± 1.3	5.2 ± 1.0
6	Untreated control[g]		7.0 ± 1.2	5.0 ± 1.2	4.5 ± 0.2	6.0 ± 0.5

[a] Mean ± S.E.
[b] $p \leq 0.05$ *versus* combined vehicle control.
[c] $p \leq 0.01$ *versus* combined untreated control.
[d] $p \leq 0.05$ *versus* combined untreated control.
[e] $p \leq 0.01$ *versus* combined vehicle control.
[f] Combined vehicle control (mean labeling index of 24 mice), 5.7 ± 0.4.
[g] Combined untreated control (mean labeling index of 24 mice), 4.8 ± 0.4.

cells 24 to 48 hr after DMH. Examination of sections, stained with acridine orange, of the distal colon of treated animals did not show any differences in the number or distribution of differentiated cells along the length of the crypt when compared with controls. Also, no labeled or damaged cells were observed in the lumen.

Proximal Colon. A single dose of i.r. DMH (0.25 to 250 mg/kg) did not seem to have any effect on the prelabeled DNA-synthesizing cells in the proximal colon or on the labeling indices 24 hr to 2 weeks afterward (Tables 3 and 4). There was no difference in the combined labeling between the vehicle and untreated controls.

Liver and Kidney. Toxic changes were seen in the liver 2 hr following administration of DMH, 250 mg/kg. Microscopically, there was marked centrolobular degeneration, vacuolization, pallid cytoplasm, and enlarged nuclei. Periodic acid-Schiff staining did not reveal any glycogen depletion. Recovery by the liver from the DMH effect was complete by 24 hr. No pathological changes were seen in the kidneys of treated animals.

DISCUSSION

We have observed that DMH at 250 mg/kg specifically diminishes the number of prelabeled DNA-synthesizing cells in the distal colon within minutes after i.r. administration and increased [^{3}H]thymidine incorporation 24 hr to 2 weeks later. At lower doses (0.25 to 25 mg/kg) the same trend was observed but less dramatically. DMH had no effect on the prelabeled DNA-synthesizing cells or labeling indices in the cells in the proximal colon, a control site under our operating conditions. The i.r. administration of PBS (vehicle control) appears to cause a slight but not significant increase in labeling indices compared to the untreated controls.

The prelabeling technique has been widely used to study

Table 3

Effects of a single i.r. instillation of DMH on prelabeled DNA-synthesizing cells in the proximal colon

[^{3}H]Thymidine was injected i.p. 30 min before, and the animals were sacrificed at 0.5, 2, and 4 hr after, DMH or vehicle instillation. Crypts in longitudinal section were measured and divided into 3 equal parts. Labeling indices in the lower and middle one-third of the crypts are presented. there was no labeling observed in the upper one-third of the crypts.

Group	Dosage (mg/kg)	Labeling indices		
		0.5 hr	2 hr	4 hr
1	0.25	2.4 ± 1.2[a]	5.0 ± 0.5	4.4 ± 0.1
2	2.5	3.8 ± 1.3	2.7 ± 1.5	5.7 ± 1.7
3	25	1.8 ± 0.5	3.8 ± 1.3	7.0 ± 1.2
4	250	6.7 ± 5.7	3.1	4.8 ± 0.5
5	Vehicle control[b]	2.7 ± 1.0	5.5 ± 1.1	3.6 ± 1.0
6	Untreated control[c]	2.9 ± 0.7	3.6 ± 1.7	3.6 ± 0.5

[a] Mean ± S.E.
[b] Combined vehicle control (mean labeling index of 24 mice), 5.9 ± 0.6.
[c] Combined untreated control (mean labeling index of 24 mice), 4.8 ± 0.4.

Table 4

Effects of a single i.r. instillation of DMH on cell proliferation in the proximal colon

[^{3}H]Thymidine was injected i.p. 1 hr before the animals were sacrificed at 24 hr, 48 hr, 72 hr, 1 week, and 2 weeks after DMH or vehicle instillation. Labeling indices in the lower and middle one-third of the crypts in longitudinal section are presented here. there was no labeling in the upper one-third of the crypts.

Group	Dosage (mg/kg)	Labeling indices				
		24 hr	48 hr	72 hr	1 wk	2 wk
1	0.25	6.4 ± 1.5[a]	8.6 ± 3.4	7.3 ± 2.5	6.8 ± 0.8	6.2 ± 1.5
2	2.5	4.7 ± 0.6		9.2 ± 1.1	8.5 ± 3.9	7.4 ± 1.5
3	25	5.2 ± 0.2	11.3 ± 4.5	9.9 ± 4.0	7.4 ± 0.2	7.1 ± 1.2
4	250	11.8	8.3 ± 1.7	5.0 ± 0.7	9.5 ± 3.2	9.4 ± 2.0
5	Vehicle control[b]	6.9 ± 1.3	7.9 ± 1.0	7.3 ± 1.5	5.1 ± 2.0	8.5 ± 1.5
6	Untreated control[c]	5.8 ± 0.1	5.6 ± 1.2	3.8 ± 0.3	5.9 ± 1.0	6.8 ± 1.3

[a] Mean ± S.E.
[b] Combined vehicle control (mean labeling index of 24 mice), 5.9 ± 0.6.
[c] Combined untreated control (mean labeling index of 24 mice), 4.8 ± 0.4.

the cytotoxic effect of drugs on DNA-synthesizing cells in the intestine and other tissues (6, 7) and is decidedly more sensitive than are morphological criteria such as pyknosis. This technique involves a pulse label with [^{3}H]thymidine followed by a test chemical 30 to 60 min later. With the pulse labeling technique, [^{3}H]thymidine is available to the DNA-synthesizing cells for a brief period of time; the unincorporated pyrimidine precursor is completely "catabolized" within 30 min (1). Quastler and Sherman (15) demonstrated that the labeling of epithelial cells in the intestine is complete within 10 to 20 min. Thus, the population of cells continues to progress through the cell cycle but the number of labeled cells remains fixed during the 4-hr period examined. We administered DMH 30 min after a pulse label and noticed a drop in the number of labeled cells within 4 hr. This suggested that the newly synthesized DNA was being destroyed rather than that DMH interferes with [^{3}H]thymidine incorporation.

Twenty-four hr after DMH instillation, cell proliferation heightened, as witnessed by the increase in the labeling index. This may be the result of DNA repair and/or compensatory synthesis, a typical phenomenon observed in target cells following a carcinogen pulse (16). This process continued for at least 2 weeks. However, the deleterious effect of a single DMH instillation seems to have disappeared within 24 hr.

Löhrs *et al.* (9) observed detectable cytotoxic damage to the epithelial cells in mice at 8 to 24 hr after a single s.c. injection of DMH. We also noted a certain degree of apparent deviation from normal crypt morphology in the DMH-treated mice. However, similar changes were also observed in vehicle only and untreated control mice, although possibly not to the same degree. These changes may be due to normal processes of tissue degeneration and regeneration and are unrelated to the effect of DMH. Thurnherr *et al.* (21) also did not observe any morphological changes in the distal colon following repeated s.c. injections of DMH to mice after 5 weeks.

However, Löhrs *et al.* (9) observed DNA-synthesizing cells in the upper one-third of the crypt after a single s.c. injection of DMH. In rats (19) and also in mice (21), repeated doses of DMH caused a loss of differentiated cells and the appearance of DNA-synthesizing cells in the upper one-third of the crypts after 5 weeks. These investigators sug-

gested that DMH distorts the preprogrammed movement of differentiated cells along the crypts. On the basis of the acridine orange stain, we found no changes by 2 weeks in the number or amount of differentiated cells in the upper two-thirds of the crypt. During this period, no DNA-synthesizing cells were seen in the upper one-half of the crypt or in the surface epithelium.

The difference in results reported by Löhrs *et al.* and by us could be due to different routes of administration. With the i.r. route of administration, the colon mucosa was bathed in the DMH solution for a short period of time, as the solution in the colon was quickly excreted or absorbed. Therefore, the effect of DMH could not be long lasting. With the s.c. route of administration, the injected DMH is slowly released into the circulation, although at a lower concentration. Therefore, the colon mucosal tissues could be exposed to the active metabolite of DMH for a longer period of time. As a result, the effects of DMH could be cumulative.

Introduced via the i.r. route of administration, DMH is absorbed through the large bowel and thus affects the liver. The changes observed in the centrolobular cells of the liver closely resemble those induced by dimethylnitrosamine (G. Williams, personal communication) and are indicative of a substance that is being actively metabolized or detoxified by the liver (10, 22).

If a putative active metabolite of DMH is released into the gut via the bile in appreciable amounts, the DNA-synthesizing cells in the proximal colon should be affected to the same extent as the cells in the distal colon. However, the toxic effect of DMH was not observed in the proximal colon (Tables 3 and 4). This is in conformity with observations that isotope from a s.c. dose of labeled DMH appears in only small amounts in bile (5, 10). The metabolic changes in the liver lead to temporary morphological damage. This may represent reversible toxic effects, since they are noted at 2 hr and disappear entirely by 4 hr.

Since it is highly unlikely that DMH could enter the liver, be metabolically activated, excreted through the bile, acted on by colon bacteria, and reach the distal colon within 30 min, it is improbable that the 30 min decrease in prelabeled DNA-synthesizing cells that we have observed is due to such a sequential metabolic activation. Instead, our data suggest that mucosal cells may possess a specific enzymatic capability to activate DMH directly. The metabolic product of the enzymatic reaction may subsequently affect DNA-synthesizing cells. In contrast, after s.c. injection of DMH, no pathological changes were found at the site of injection (4, 19, 21, 22). It is probable that the cells at the s.c. site do not possess the enzymes capable of reacting with DMH. Hence, s.c. administered DMH may reach the colon tissue unchanged through the circulation, to be activated at the site by the intestinal cells, a concept that we are currently investigating. In light of the present findings, the potent carcinogenic effect of a weekly dose of DMH might result from repeated challenge to the DNA-synthesizing cells in the crypts of Lieberkühn at a time of heightened or compensatory DNA synthesis.

ACKNOWLEDGMENTS

We are grateful to Dr. M. Lipkin of the Sloan-Kettering Memorial Cancer Center, New York, and Dr. G. M. Williams of this Institute for useful discussions.

REFERENCES

1. Baserga, R., and Malamud, D. Autoradiography Technique and Application, p. 151. New York: Harper and Row Publishers, 1969.
2. Chan, P. C., Johnson, L. I., Monette, F. C., LoBue, J., and Gorden, A. S. Tritiated-thymidine Incorporating Cells in the Peripheral Blood of Normal and Splenectomized Rats. Proc. Soc. Exptl. Biol. Med., *125:* 614–617, 1967.
3. Cohen, L. A. Some Ultrastructural, Biochemical and Physiological Changes Induced by the Carcinogen 4-Nitroquinoline *N*-Oxide in the Flagellate, *Crithidia fasciculata*. Ph.D. Dissertation, City University of New York, 1972.
4. Druckrey, H. Organospecific Carcinogenesis in the Digestive Tract. *In:* W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima (eds.), Topics in Chemical Carcinogenesis, pp. 73–104. Baltimore: University Park Press, 1972.
5. Fiala, E. S., and Weisburger, J. H. Metabolism of 1,2-Dimethylhydrazine-^{14}C. Proc. Am. Assoc. Cancer Res., *16:* 15, 1975.
6. Hennings, H. Hydroxyurea: Possible Relationship of Cytotoxicity to Inhibition of Skin Carcinogenesis. J. Natl. Cancer Inst., *46:* 841–844, 1971.
7. Lenaz, L., Sternberg, S. S., and Philips, F. S. Cytotoxic Effects of 1-β-D-Arabinofuranosyl-5-fluorocytosine and of 1-β-D-Arabinofuranosylcytosine in Proliferation Tissues in Mice. Cancer Res., *29:* 1790–1798, 1969.
8. Lipkin, M. Proliferative Changes in the Colon. Am. J. Digest. Diseases, *19:* 1029–1032, 1974.
9. Löhrs, M., Wiebecke, B., and Eder, M. Morphologische und Autoradiograpische Untersuchung der Darmschleimhautveränderungen nach einmaliger Injektion von 1,2-Dimethylhydrazin. Z. Ges. Exptl. Med., *157:* 297–307, 1969.
10. Magee, P. N., Hawks, A., Stewart, B. W. and Swann, P. F. Mechanisms of Carcinogenesis by Alkylnitrosamines and Related Compounds. *In:* Pharmacology and the Future of Man. Proceedings of the Fifth International Congress on Pharmacology, San Francisco, 1972, pp. 140–149. Basel: S. Karger AG, 1973.
11. Narisawa, T., Sato, T., Hayakawa, M., Sakuma, A., and Nakano, H. Carcinoma of the Colon and Rectum of Rats by Rectal Infusion of *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine. Gann, *62:* 231–234, 1971.
12. Narisawa, T., and Weisburger, J. H. Colon Cancer Induction in Mice by Intrarectal Instillation of *N*-Methylnitrosourea. Proc. Soc. Exptl. Biol. Med., *148:* 166–169, 1975.
13. Narisawa, T., Wong, C. Q., and Weisburger, J. H. Induction of Carcinoma of the Large Intestine in Guinea Pigs by Intrarectal Instillation of *N*-Methyl-*N*-nitrosourea. J. Natl. Cancer Inst., *54:* 785–787, 1975.
14. Pegg, A. E., and Hawks, A. Increased Transfer Ribonucleic Acid Methylase Activity in Tumors Induced in the Mouse Colon by the Administration of 1,2-Dimethylhydrazine. Biochem. J., *122:* 121–123, 1971.
15. Quastler, H., and Sherman, F. G. Cell Population Kinetics in the Intestinal Epithelium of the Mouse. Exptl. Cell Res., *17:* 420–438, 1959.
16. Rajewsky, M. F. Proliferative Parameters of Mammalian Cell Systems and Their Role in Tumor Growth and Carcinogenesis. Z. Krebsforsch. Klin. Onkol., *78:* 12–30, 1972.
17. Reddy, B. S., Narisawa, T., Wright, P., Vukusich, D., Weisburger, J. H., and Wynder, E. L. Colon Carcinogenesis with Azoxymethane and Dimethylhydrazine in Germ-free Rats. Cancer Res., *35:* 287–290, 1975.
18. Reddy, B. S., Weisburger, J. H., Narisawa, T., and Wynder, E. L. Colon Carcinogenesis in Germ-free Rats with 1,2-Dimethylhydrazine and *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine. Cancer Res., *34:* 2368–2372, 1974.
19. Springer, P., Springer, J., and Oehlert, W. Die Vorstufen des 1,2-Dimethylhydrazin-induzierten Dick-und Dünndarmcarcinoms der Ratte, Z. Krebsforsch, *74:* 236–240, 1970.
20. Thompson, S. W., and Hunt, R. D. Selected Histochemical and Histopathological Methods, p. 269. Springfield, Ill.: Charles C Thomas, 1966.
21. Thurnherr, N., Deschner, E. E., Stonehill, E. H., and Lipkin, M. Induction of Adenocarcinomas of the Colon in Mice by Weekly Injections of 1,2-Dimethylhydrazine. Cancer Res., *33:* 940–945, 1973.
22. Weisburger, J. H. Chemical Carcinogens and Their Mode of Action in Colonic Neoplasia. Diseases Colon Rectum, *16:* 431–437, 1973.