The Effect of Halothane on the Cell Cycle in Rat Small Intestine

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One hundred thirty rats were given 250 microcuries of tritiated thymidine (^{3}HT) then exposed to air or to 0.1 or 0.5 per cent halothane in air for ten minutes to 24 hours Radioautographs of small-bowel epithelial cells were made and the percentages of labeled mitotic figures determined. From these data, graphic estimation of the duration of DNA synthesis in the cell cycle gave the following results: air, 6.1 hours; 0.1 per cent halothane, 7.9 hours; 0.5 per cent halothane, 10.2 hours. Exposure of animals to halothane for two or 12 hours prior to injection of ^{3}HT did not alter the percentages of mitotic figures labeled two hours after giving the isotope. The effect of halothane on these cells is to prolong DNA synthesis; other phases of the cell cycle apparently are unaffected.

MORE THAN 50 YEARS AGO, it was known that anesthetics inhibit cell division. Despite the recent growth of knowledge of cell biology, the reason for this inhibition remains unknown. Without this basic knowledge it is difficult to interpret studies such as those concerning possible teratogenicity of anesthetic agents.[1,2,3]

We now know that dividing cells go through the following sequential steps: division (M phase); postmitotic rest (G1 phase); DNA synthesis, doubling the nuclear content of this material (S phase); a brief resting phase (G2) before once again dividing. Radioactive thymidine, a specific DNA precursor, may be given to dividing cells and the time course of these phases of the cell cycle followed. Using this technique, we reported a pilot study of the effects of halothane on rat small bowel epithelium.[4] At that time, we thought DNA synthesis was not affected by halothane. The present report, based on a series of 146 rats, revises that conclusion and shows clearly that halothane prolongs the period of DNA synthesis without affecting the mitotic act itself.

Methods

The methods used in this investigation were essentially the same as those described previously.[4] Male and female Sprague-Dawley rats weighing between 200 and 260 g were housed for a minimum of a week prior to use. They were fed standard rat diet and water *ad lib.* except during the experimental exposure. Immediately after injection with 250 microcuries of thymidine-methyl-^{3}H (^{3}HT, specific activity 2 to 4 c/mM) intraperitoneally, the rats were placed in a 15-l plastic box. Ten liters per minute of air containing 0, 0.1 or 0.5 per cent halothane circulated through the box. The rats slept if undisturbed while receiving 0.1 per cent halothane, and were fully anesthetized by the 0.5 per cent concentration. Halothane and CO_2 concentrations were monitored by means of a gas chromatograph. At specified intervals (see table 1), rats were taken from the box, killed by cervical dislocation, and tissues rapidly removed and fixed in acetic-alcohol-formalin. Tissues were stored overnight at 2 C, changed to absolute alcohol the next day, and 4-micron-thick sections prepared for radioautography.[5] Sections were dipped in Kodak NTB-2 radioautography emulsion, exposed in the dark for 14 days, developed and stained with hematoxylin and eosin. Epithelial cells of the crypts were examined microscopically and 100 consecutive mitotic figures were

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TABLE 1. Mean Percentages of Epithelial Crypt Cells Labeled at Intervals Following Injection of ^{3}HT and Subsequent Exposure to Air or 0.1 or 0.5 Percent Halothane in Air

Time (hours)	Treatment								
	Air			0.1 Per cent Halothane			0.5 Per cent Halothane		
	No. of Rats	Mean	SE	No. of Rats	Mean	SE	No. of Rats	Mean	SE
0	2	0.0	—	—	—	—	2	0.0	—
1	4	31.8	2.2	—	—	—	5	13.4	2.2
2	4	96.0	1.0	2	95.5	1.1	4	94.8	0.7
4	4	97.3	0.8	4	96.5	0.7	6	97.3	1.5
6	6	90.0	2.5	6	94.2	1.2	6	97.5	1.6
8	6	29.0	4.6	6	63.2	4.0	6	91.2	4.3
10	4	73.8	3.6	3	41.0	10.4	6	51.7	12.5
12	4	80.5	3.3	4	66.0	4.0	6	48.3	10.8
15	3	84.0	2.7	4	75.5	3.2	4	58.0	3.7
18	4	91.2	1.6	3	81.7	5.8	4	73.2	13.5
24	4	86.2	0.5				4	75.2	9.7

inspected for the presence of radioactive label. No correction for background grain count was necessary. Results were expressed as the per cent of mitotic figures labeled per animal. In this series, the mitotic index was computed for eight rats which had received either air or 0.5 per cent halothane for eight hours. This was done by counting the total number of mitotic figures (with or without label) observed in sections of 10 crypts of Lieberkühn for each animal.

Tracer injection and air or halothane treatment were begun simultaneously in 130 rats. In contrast, 16 rats were pretreated with inhalation of air containing 0, 0.1 or 0.5 per cent halothane for periods of either two or 12 hours prior to intraperitoneal injection of 250 μc of ^{3}HT. Air or halothane treatment was then continued for two hours after injection, the animals killed and radioautographs prepared.

Results

The results are summarized in tables 1 and 2. To analyze the cell cycle the data in table 1 were plotted in figure 1. The initial appearance of labeled mitotic figures occurred at the same rate in the control and anesthetized rats, but the descending limb of the curve showed a progressively shallower slope as the concentration of halothane increased. Consequently, the DNA synthesis time (S phase) showed a progressive increase as the concentration of the anesthetic increased. The mean duration of the S period, estimated as that length of time between the ascending and the descending limbs of the curve crossing the 50 per cent point of labeling, was 6.1 hours for air-treated animals, 7.9 hours for the 0.1 per cent halothane group and 10.2 hours for 0.5 per cent halothane treatment. The data from the halothane-treated animals showed more variation, especially at the ten-hour interval, as indicated by the standard errors in figure 1. Variation was greatest in the 0.5 per cent halothane experiment, while that of the controls was least. This suggests that there are degrees of susceptibility to halothane among proliferating crypt cells. The mitotic indexes of the control and 0.5 per cent halothane-treated rats did not differ at eight hours.

The results from the 16 rats pretreated with air or one of the two concentrations of halothane prior to the injections of ^{3}HT, then continued for two hours prior to removal of tissue, are given in table 2. Pretreatment for these periods did not alter the percentages of mitotic figures labeled at two hours after ^{3}HT injection, and the numbers are virtually identical with those obtained in the first experiment.

Discussion

The data clearly indicate that halothane prolongs the time required for DNA synthesis in the small-bowel epithelial cells of the rat

and virtually rule out any effect on the G1, G2 or mitosis phase of the cell cycle. To put this into perspective, a brief summary of the interpretation of such findings is offered.

The cell cycle consists of two phases: interphase, which has been further subdivided into G1, S and G2; and mitosis, which consists of prophase, metaphase, anaphase and telophase. Until recently, the events of interphase were little understood. Biologists studying cell division concentrated attention on the four recognizable phases of mitosis.

Howard and Pelc, in 1953, studied cell proliferation with radioautography and ^{32}P.[6]

TABLE 2. Results from Rats Exposed Both before and after ^{3}HT Injection

No. of Rats	Halothane Concentration (Per cent)	Duration of Inhalation before ^{3}HT (hours)	Duration of Inhalation after ^{3}HT (hours)	Per cent of Crypt-cell Mitotic Figures Labeled (Mean)
2	0.0	2	2	95
3	0.1	2	2	92
2	0.5	2	2	94
3	0.0	12	2	96
3	0.1	12	2	93
3	0.5	12	2	94

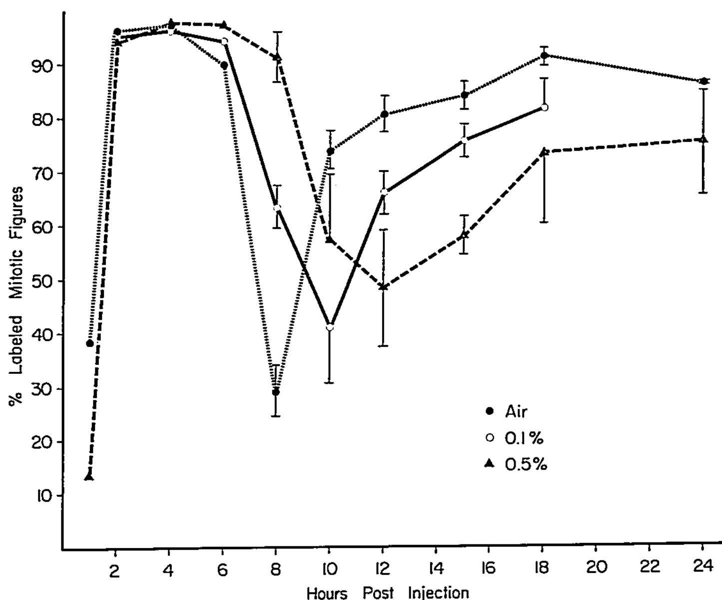

FIG. 1. Percentages of small-bowel epithelial crypt cell mitotic figures which contained a radioactive label at intervals following injection of 250 μc ^{3}HT and subsequent inhalation of air or 0.1 or 0.5 per cent halothane in air. Bars indicate ±1 SE except at points where one bar was omitted to avoid overlap with another from an adjacent point. Data for this graph are tabulated in table 1. DNA synthesis time (S period) is that time interval between ascending and descending curves crossing the line of 50 per cent labeled mitotic figures.

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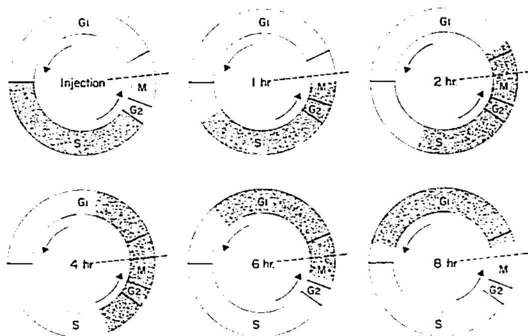

FIG. 2. Diagrammatic representation of phases of the cell cycle, showing initial uptake of ³HT into S phase cells at time of injection of tracer, and passage of this labeled block of cells through mitosis (M phase) with time. Dashed line through M segment of the cycle indicates halfway mark, the point of reference through which the labeled cells pass. The M phase is enlarged to show clearly the passage of labeled cells through it. (Figure modified, with permission of author and publishers, from Thrasher, J. D.: Analysis of renewing epithelial cell populations. *In* Prescott, D. H. (ed.): Methods in Cell Physiology. New York, Academic Press, 1969, p. 336.)

On the basis of their investigation, interphase was divided into three periods: the period of DNA synthesis, called the S phase; the gaps in activity preceding and following the S phase (*i.e.*, immediately after and immediately before mitosis) were designated G1 and G2, respectively. This terminology is standard today, and has been applied to many studies using thymidine (a specific DNA precursor) with a tritium label.

The most commonly used technique for studying phases of the cell cycle utilizes labeled mitoses.[7] The method employed in our study is standard, often referred to as "pulse labeling," wherein a dose of ³HT is given intraperitoneally. This substance is rapidly taken up by cells in the process of DNA synthesis during interphase, and the uptake and distribution of ³HT following intraperitoneal injection is complete within 10 to 20 minutes. Thus, the method involves labeling a block of cells, those in the S period, and observing the movement of this block through mitosis, a fixed point in the cell cycle. These stages are shown in figure 2.

It is important to understand, when analyzing figure 1, that the curves do not represent the actual number of mitoses. The mitotic index, *i.e.*, the relative number of cells in mitosis at any given moment, appears to be unaffected by 0.1 or 0.5 per cent halothane. The curves indicate the fraction of those cells in mitosis which carry a DNA label. Regardless of the concentration or duration of administration of halothane, virtually 100 per cent of the mitotic figures were labeled two hours after injection of ³HT. This shows that the G2 phase and mitosis were not prolonged by halothane. Prolongation of these phases would increase the time taken by labeled cells to reach the point of 100 per cent labeled mitotic figures. Tables 1 and 2 show no differences among the control and various treatment groups. The progressive lags in the descending limbs of the curves for increasing concentrations of halothane, shown in figure 1, indicate a slowing of cells moving through the S phase. Thus, we conclude that halothane specifically increases the period of DNA synthesis in these cells.

These curves are quite similar to those derived from a study of the effects of aging on the cell cycle.[8] However, they differ distinctly from those published in conjunction with reports of the effects of colchicine.[9] This is important in relation to studies based on the "C-mitosis" theories published by Ostergren[10] and, more recently, by Andersen.[11] They concluded that there was a similarity between the effect of anesthetics on cell division and that of colchicine, an arrester of mitosis at metaphase. Our data do not support this theory, and an additional observation may be made to emphasize this. Leblond and Stevens,[12] in stud-

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ies of the dynamics of cellular renewal in the intestinal epithelium of the rat, showed that this tissue renewed itself at the same rate in male and female, young and old, and at different times of the day. At any given time, 3.5 per cent of the cells in the epithelial crypts of Lieberkühn of the ileum were in the act of mitosis. Over a period of 32.4 hours these crypt cells divided, migrated to the tip of the intestinal epithelial villus, and were shed into the lumen of the bowel. Since the villus cells do not synthesize DNA or divide, labeled epithelial cells on the villus must be the progeny of the dividing cells in the crypts which migrated upward with time. Had halothane arrested cells in metaphase, labeled metaphase figures would have been observed on the villi of the rats studied 12 to 24 hours after injection of ^{3}HT. This was not observed in our preparations.

Our data suggest that halothane has no effect on the "gaps" of interphase known as G1 and G2. A recent report[13] of the effect of amethopterin on the cell cycle of cells *in vitro* indicates that inhibition of DNA synthesis by this agent does not alter the constancy of the G2 period which follows. It appeared to these workers that complete reduplication of DNA must be concluded before the onset of G2, which then proceeds normally whether or not DNA synthesis has been inhibited. We conclude from our studies that halothane has a similar effect on the proliferating cells of the rat small bowel. Although these studies demonstrate an effect on a specific process of cellular nucleoprotein metabolism, they do not elucidate the mechanism.

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