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PROLIFERATION KINETICS OF TUMOR AND HOST CELLS AFTER CHEMOTHERAPEUTIC TREATMENT

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ABSTRACT

In order to find the most effective schedule of Mitomycine C (MMC) treatment against tumor bearing individuals, an investigation was made to compare the growth kinetics of solid and ascites tumor cells with that of intestinal mucosa cells before and after MMC treatment. In the duodenal crypt epithelium of the mouse, desoxyribonucleic acid (DNA) synthesis as measured by thymidine uptake was first depressed within 0.5 hours after a single injection of 40 μg/kg or 400 μg/kg of MMC, significantly increased over the control level at 24 hours and then gradually returned to the range of the control. Similar results were obtained in mitotic activity. In contrast, DNA synthesis in Ehrlich ascites tumor was depressed within 0.5 hours and not recovered yet at 24 hours after the same dosage of MMC. It was also noted that the cell repair in Yoshida solid tumor, after the damage due to MMC, was slow as compared to that in the intestinal mucosa of the rat. These data suggest that in the duodenal crypt epithelium some compensatory homeostatic mechanism of tissue level, which may alter their proliferative state, may be operative after the administration of the chemotherapeutic agents. It is, therefore, recommended that antitumor agents should be given under consideration of the different kinetics of regulatory cell repair of host and tumor tissues.

INTRODUCTION

Action of most antitumor agents, which are clinically used, is to interfere with some stages of nucleic acid or protein synthesis. The significant differences, which would provide the key to cancer chemotherapy, have not been found between normal and tumor cells in the biosynthesis of nucleic acid and protein. They are, therefore, selectively cytotoxic to rapidly dividing cells rather than tumor cells. Since normal cells, such as the epithelial cells of small intestine or the cells of bone marrow, have a shorter cell cycle than most tumor cells, antitumor agents will be as effective on those rapidly dividing normal cells as on most tumor cells. Normal tissue cells are, however, different from tumor cells in cell population recovery. That is, the cell recovery of normal tissues may be well regulated by

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homeostasis as compared with tumor cells. Accordingly a fundamental question in cancer chemotherapy is whether the relative effects of a drug on normal and tumor cells are constant under all circumstances or the relationship may be altered by the schedule of treatment. Such factors as the time of initial treatment, total dosage, the duration of treatment and the interval between administration of individual doses may influence the host-tumor relationship. These factors should be taken into consideration in determining the usefulness of antitumor agents.[1)2)3)4)] This investigation was undertaken to answer the question whether the usefulness of antitumor agents is influenced by the interval between administration of individual doses.

MATERIALS AND METHODS

Male dd mice weighing approximately 20 g and male Wister rats weighing approximately 100 g were used. Ehrlich asictes tumor cells and Yoshida sarcoma cells used in this experiment were maintained in the form of ascites in the abdominal cavity of dd mice and Wister rats respectively. MMC was provided from Kyowa Fermentation Industry, Ltd.

Mice received 800 μg/kg of MMC in split administration with 24 hour's interval (400 μg/kg each time), which was repeated every 3 days 10 times (8000 μg/kg total dose). The other mice received 800 μg/kg or 400 μg/kg of MMC in a single administration, which was also repeated every 3 days 10 times (8000 μg/kg or 4000 μg/kg total dose). The toxicity of MMC in each group was compared. On the other hand, mice were inoculated with 300×10^4 Ehrlich ascites tumor cells. Each mouse bearing 4 day old tumor started to receive 800 μg/kg of MMC in split administration, and 800 μg/kg and 400 μg/kg of MMC in a single administration. The survival time of each group was compared. In these experiments 10 mice were used for each group.

Mice and rats were given tritiated thymidine (Daiichi Chemical Campany, Ltd; specific activity of 12 mC/m mole) in a dose of 1 μC per g body weight by intraperitoneal injection at 0.5, 3, 6, 12, 24 and 72 hours after administration of MMC, and killed by cervical dislocation 30 minutes later. The duodenum (a 2 cm long segment 2 cm distal to the pyloduodenal junction) was excised immediately after the death of the mice or the rats. The tissues were homogenized with a Potter-Elvejem homogenizer in ice-cold phosphate buffered saline solution (PBS). The homogenates were precipitated in cold 10% trichloroacetic acid (TCA), the acid-soluble radioactivity was extracted by resuspending the precipitates in three successive portion of cold TCA and the precipitates were then defatted with ethanol-ehter (3:1). The residues were hydrolyzed with hot 10% TCA for 15 minutes at 90° to obtein DNA fraction.[5)] After 0.2 ml of the extracted DNA solution was mixed in 10 ml dioxan scintilater[6)] (Naphthalin 100 g: DPO 10 g: POPOP 250 mg: Dioxan 1 L), radioactivity was counted with Ten liquid scitilation counter. The quantity of DNA was measured by Burton's method.[7)] Specific activity was

calculated by the ratio of DNA counts (dpm) to the quantity of DNA (μg). For microscopic study, the duodenum was removed after the treatment as described above, fixed in 10% formalin, and sections, 4 μ in thickness, were cut parallel to the longitudinal axis of the intestine. The slides were dipped in Sakura NRM2 emulsion, exposed, developed in Konidol developer and stained with hematoxylin and eosin. Exposure time ranged from 3 to 4 weeks. Labeled cells and mitotic cells were counted in the epithelium of 20 duodenal crypts for determination of labeling index and mitotic index respectively. On the other hand, mice were inoculated intraperitoneally with 300×10^4 Ehrlich ascites tumor cells. Each mouse bearing 4 day old tumor was given tritiated thymidine at 0.5, 24 and 72 hours after administration of MMC, and killed 30 minutes later. Ehrlich ascites tumor cells were withdrawn into PBS solution immediately after the death of the mice. For measurement of the specific activity, the tumor cells were treated as described above. For microscopic study, the tumor cell smears were made on clean slides, dried and fixed in absolute alcohol for 15 minutes. The slides were dipped in Sakura NRM2 emulsion, exposed, developed and stained. Labeled cells and mitotic cells were counted in 1000 total cells for determination of labeling index and mitotic index respectively. Rats were inoculated subcutaneously with 100×10^4 Yoshida sarcoma cells. The rats bearing 5 day old tumor were injected with MMC intraperitoneally and killed 6, 24, 72 and 120 hours later. In the first group, tritiated thymidine was injected intraperitoneally at 30 minutes before administration of MMC, and in the second group, tritiated thymidine was injected at 30 minutes before sacrifice. The tumors were subjected to prepare parafin section for autoradiography. In these experiments 3 mice or rats were used for each time point. The dosage of MMC is listed with the individual experiments. All doses were administered by intraperitoneal injection in a volume of 0.5 ml per mouse or rat, and the control mice or rats received saline in a corresponding volume.

RESULTS

Toxicity of MMC

Fig. 1 represent the toxicity of MMC in mice characterized by weight loss. The mean body weight of the mice which were treated with the splitting way administration of 800 μg/kg of MMC for 10 times every 3 days (8000 μg/kg total dose) decreased from 20.6 g to 19.9 g at 37 days after the first injection, that of the mice treated with the simple way administration of 800 μg/kg of MMC (8000 μg/kg total dose) increased slightly from 20.3 g to 21.8 g, and that of the control mice increased from 20.6 g to 23.8 g. Diarrhea appeared at 15 to 18 days in the group treated with the splitting way administration of 800 μg/kg and at 20 to 24 days in the group treated with the simple way administration of 800 μg/kg after the first injection of MMC. The total dose toxicity for the mice observed in the simple way administration was less than that observed in the splitting way administration.

The suvival time of the mice inoculated with Ehrlich ascites tumor is shown in Fig. 2. The splitting way administration showed to be approximately equal in effects on the survival time to the simple way administration.

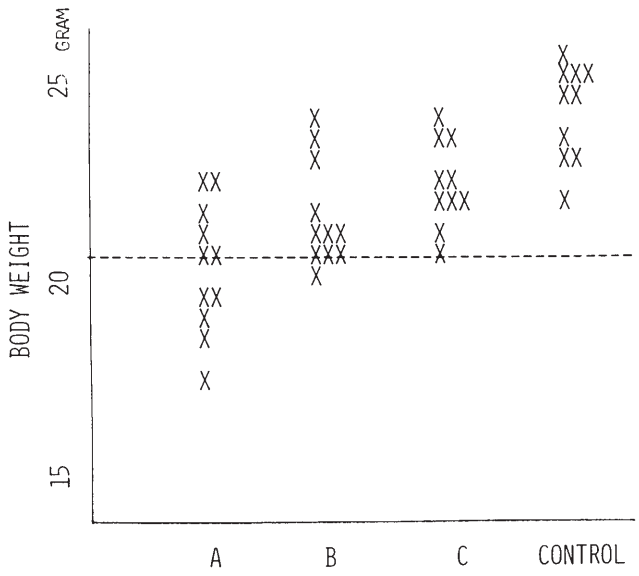


Fig. 1

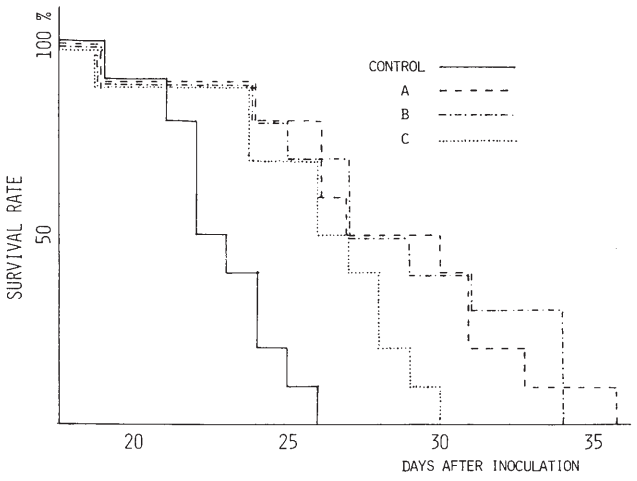


Fig. 2

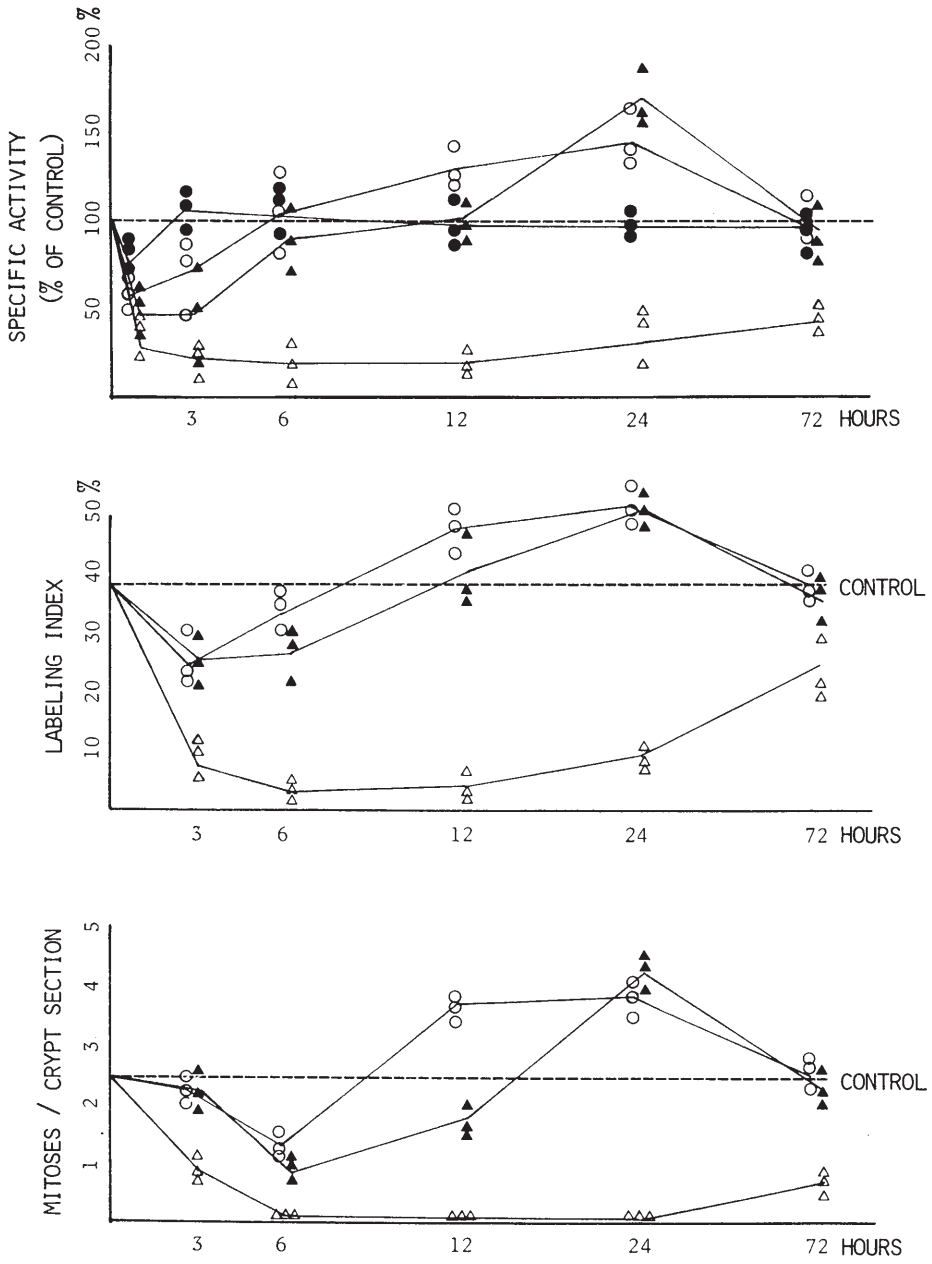
Effects of MMC on duodenal epithelial cells

After a single injection of 400 μg/kg of MMC, extensive damage was observed at 3 to 6 hours, thereafter degenerating cells steadily decreased in number, and by

24 hours only occasional degeneration cells and cell debris were present in the mouse duodenal crypt epithelium. The degenerating cells were present only in the proliferative zone of the crypt, and panet cells and the cells of the upper third of the crypt were not affected. Villar epithelial cells in the duodenum showed no sign of damage. Fig. 3 represent the DNA specific activity observed at various intervals after administration of MMC. After administration of 4 μg/kg, the specific activity was within the range of the control. After administration of 40 μg/kg and 400 μg/kg, the specific activity decreased to 87% and 49% respectively at 3 hours, reached 148% and 168% over the control at 24 hours and then gradually returned to the range of the control by 72 hours. After administration of 4000 μg/kg, the specific activity decreased progressively to 23% of the control at 12 hours and gradually returned to 61% by 72 hours. There were marked differences in response to these four doses administered. The patterns of labeling index curves (Fig. 3) showed the same trend as those of the specific activity curves. The over-shoot in the labeling index at 12 to 24 hours after administration of 40 μg/kg or 400 μg/kg shows that the over-shoot in the specific activity is caused by the increase of DNA synthesizing cells in the crypts although, to some extent, it may be caused by the loss of innert DNA (DNA from villi epithelium). The mitotic index observed at various intervals after administration of MMC is shown in Fig. 3. After administration of 40 μg/kg and 400 μg/kg, mitotic cells were significantly reduced at 6 hours, rose to values which were markedly more than those in the control at 24 hours and eventually returned to the range of the control by 72 hours. After administration of 4000 μg/kg, mitotic cells were negligible from 6 to 24 hours and only slight recovery was observed at 72 hours. The patterns of curves of specific activity, labeling index and mitotic index in the duodenal crypt epithelium of the rats after administration of MMC showed the same trend as those of the mice.[8)]

Effects of MMC on Ehrlich ascites tumor cells

After a single dose of 40 μg/kg or 400 μg/kg of MMC, degenerating cells and cell debris were yet observed microscopically in Ehrlich ascites tumor at 24 hours. Fig. 4 represents the specific activity observed at various intervals after administration of MMC. After administration of 4 μg/kg and 40 μg/kg, the specific activity decreased to 84% and 55% respectively at 24 hours and returned to the range of the control by 72 hours. After administration of 400 μg/kg and 4000 μg/kg, the specific activity markedly decreased to 49% and 8% respectively at 24 hours and no recovery occured by 72 hours. It may be partly caused by dilution of the viable cells with the degenerating cells and cell debris that the values of the specific activity in Ehrlich ascites tumor at 24 hours after administration of MMC were lower than those in the control. Results similar those of the specific activity were obtained in the labeling index (Fig. 4). The mitotic index observed at various intervals after administration of MMC is shown in Fig. 4. After administration of 40 μg/kg, mitotic cells reduced to the values which were half as many as those in


SPECIFIC ACTIVITY
(% OF CONTROL)
200 %
150
100
50
3
6
12
24
72 HOURS
LABELING INDEX
50 %
40
30
20
10
CONTROL
3
6
12
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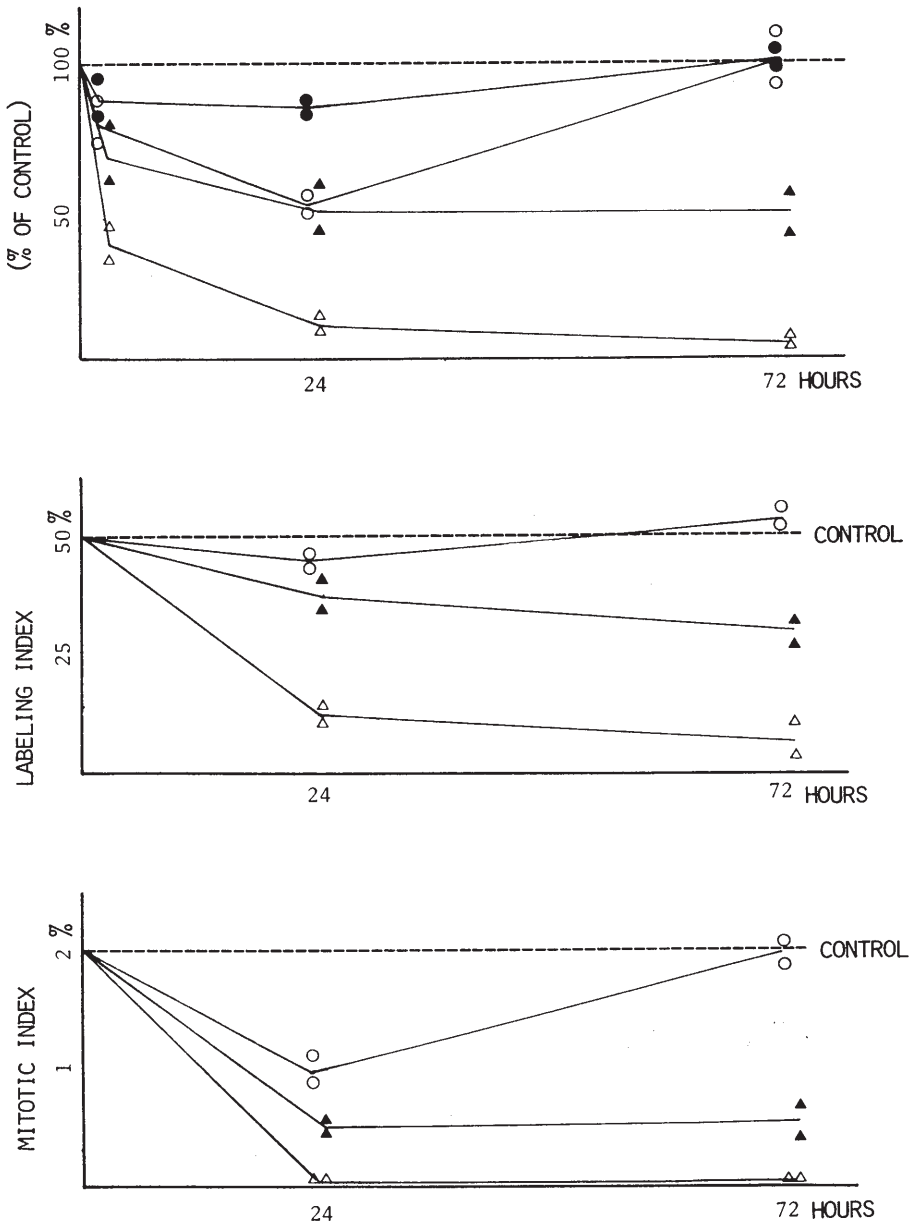
72 HOURS
MITOSES / CRYPT SECTION
5
4
3
2
1
CONTROL
3
6
12
24
72 HOURS

Fig. 3


(% OF CONTROL)
100 %
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LABELING INDEX
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CONTROL
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MITOTIC INDEX
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CONTROL
24
72 HOURS

Fig. 4

the control at 24 hours and returned to the range of the control by 72 hours. After administration of 400 μg/kg, mitotic cells were significantly reduced at 24 hours and no recovery occured by 72 hours.

Effects of MMC on Yoshida sarcoma cells

By microscopic examination, the tumor mass was grossly divided to 3 layers; (1) the central area where most cells undergo necrosis, (2) the area supplied by rich blood vessels and observed highest cell proliferation as determined by labeling index and mitotic index (A zone), and (3) the most external zone of the tumor mass where host round cell infiltration was most marked and the cell proliferation was less than that seen in A zone (B zone). Fig. 5 represents the labeling index observed at various intervals after administration of MMC. The cell damage by MMC varied depending on the localization. The degenerating cells were observed mainly at the A zone where most rapid proliferation was suggested because of the highest mitotic index and labeling index. After administration of 200 μg/kg, the labeling index in the A zone decreased to lower than 20% from 6 to 72 hours and returned to the range of the control by 120 hours. In contrast, the labeling index in the B zone remained within the range of the control throughout the course. When tritiated thymidine was injected at 30 minutes before sacrifice, the labeling index was highest in the A zone (Fig. 5). When tritiated thymidine was injected at 6 to 24 hours before sacrifice, the labeling index of the A zone was equal to that of the B zone (Fig. 6). This highly suggests that the cells proliferated in the A zone migrated to the B zone afterwards.

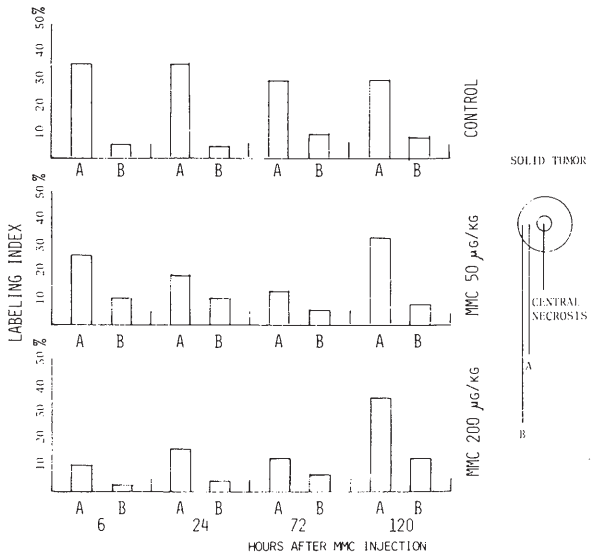

Fig. 5

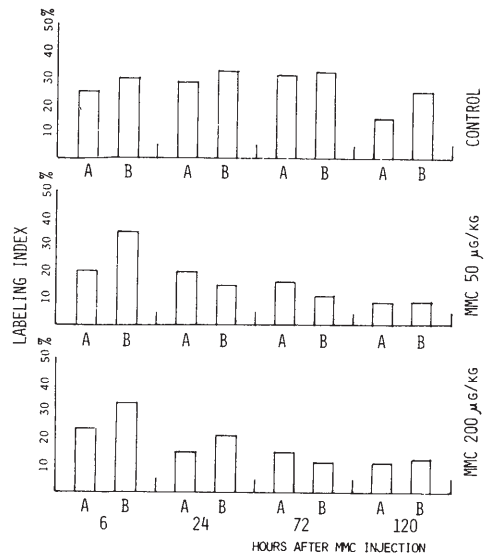

Fig. 6

DISCUSSION

The results described above demonstrate that the simple way administration of MMC were definitely less toxic to host than the splitting way administration of the same dosage, although the effects of the drug on Ehrlich ascites tumor were same among both administrations. Kenis, Y.[9] reported that the massive intermittent doses of MMC (20 to 50 mg) were definitely less toxic and probably more active than the small daily doses. Mathe, G.[10] also showed that the same total dose does not give the same results according to whether the drug is given continuously divided into small daily doses or intermittently in a massive dose. These observations suggest the possibility that there may be differences between host and tumor in the rate of recovery from the toxic effects of MMC.

Bruth, W. R. *et al* classified antitumor agents in three classes. The action of the agents of first class is independent of the cell cycle, the agents of second class act on a single phase of the cell cycle sparing the cells in other phases, and the agents of third class act on all or most phases of the cell cycle and not on the cells in G_0. MMC is an agent of third class. Accordingly, it is considered that MMC is less active on the cells which are in a non-proliferative state and more active on the cells in a proliferative state, especially in the S phase. As shown in Fig. 3, the inhibition of DNA synthesis in the duodenal crypt epithelium showed a maximum at approximately 0.5 to 3 hours after the administration of the drug, and the inhibition of mitotic activity, on the other hand, reached a maximum at approxi-

mately 6 hours for all doses. These are the results to be expected if the cells in the G_2 and M phase of the cell cycle are less affected than the cells in the S phase by MMC, since the duplication of DNA is prerequisite to mitotic division and the duration of the G_2 phase is approximately 2 hours in the crypts.

It was determined by the labeled mitosis wave method[13)14)15)] that in the duodenal crypt epithelium of the mouse the generation time was 12 hours and the duration of various stages was 2 hours in G_1, 7.5 hours in S, 2.5 hours in combined G_2 and M, and the growth fraction was 70%. On the other hand, in the 4 day old Ehrlich ascites tumor the generation time was 12 hours and the duration of various stages was 2 hours in G_1, 8 hours in S, 1.5 hours in combined G_2 and M, and the growth fraction was 70%. There were no significant differences between each cell population kinetics. In contrast, the results presented in Fig. 3 and Fig. 4 indicate that there were marked differences in the specific activity, labeling index and mitotic index between the mouse duodenal epithelial cells and Ehrlich ascites tumor cells after administration of MMC. As shown in Fig. 3, marked increase of DNA synthesizing cells was observed in the duodenal crypt epithelium at 12 to 24 hours after administration of 40 μg/kg or 400 μg/kg of MMC. It is also reported that the action of MMC[16)17)18)19)] is so rapidly terminated by its metabolic inactivation and renal excretion that its antitumor activity is correspondingly transient, lasting only so long as the effective concentration of the agent is present in tissues. From the point of biological action of MMC described above, these results suggest that some compensatory homeostatic mechanism of tissue level may be operative in the duodenal crypt epithelium after the administration of the drug. It seems, therefore, reasonable to suppose that non-dividing cells, which were not injured by MMC, may re-enter into the proliferative cycle and initiate to synthesize DNA through the operation of the homeostatic mechanism of tissue level as soon as the action of MMC terminates. In contrast, there was observed no increase of DNA synthesizing cells, as shown in Fig. 4, in Ehrlich ascites tumor cells after the administration of the drug. It was also noted, as shown in Fig. 5, that the regulatory cell repair in Yoshida solid tumor cells, after the damage due to the drug, was slow as compared to that in the rat intestinal mucosa. These results suggest that there may be few regulatory mechanism of tissue level in Ehrlich ascites tumor or Yoshida solid tumor.

From these data, it is possible to define theoretically an optimal mode of administration of MMC. As a rule, dosage limit is determined not by the effects on tumor but by the torelance of normal structures. Accordingly the first dose of MMC should be given at the highest tolerated dose and the second dose should be given after an interval sufficient to allow the host cells to return to the cells of steady state, so-called cells in the G_0 phase of cell cycle which escape injury and serve to repopulate the proliferating tissues after the disappearance of the drug.[20)]

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LEGENDS TO FIGURES

FIG. 1. The toxicity of MMC in mice characterized by weight loss. A, 800 μg/kg of MMC in the splitting way administration (8000 μg/kg total dose); B, 800 μg/kg of MMC in the single way administration (8000 μg/kg total dose); C, 400 μg/kg of MMC in the single way administration (4000 μg/kg total dose).

FIG. 2. The survival time of the mice inoculated with Ehrlich ascites tumor. A, 800 μg/kg of MMC in the splitting way administration (1600 μg/kg total dose); B, 800 μg/kg of MMC in the single way administration (1600 μg/kg total dose); C, 400 μg/kg of MMC in the single way administration (800 μg/kg total dose).

FIG. 3. The effects of different doses of MMC on DNA specific activity, labeling index and mitotic activity in the mouse duodenal crypt epithelium. Each point is the result from a single animal. ●, 4 μg/kg; ○, 40 μg/kg; ▲, 400 μg/kg; △, 4000 μg/kg.

FIG. 4. The effects of different doses of MMC on DNA specific activity, labeling index and mitotic index in Ehrlich ascites tumor cells. Each point is the result from a single animal. ●, 4 μg/kg; ○, 40 μg/kg; ▲, 400 μg/kg; △, 4000 μg/kg.

FIG. 5. The effects of MMC on solid Yoshida sarcoma cells. Tritiated thymidine was injected 30 minutes prior to sacrifice.

FIG. 6. The effects of MMC on solid Yoshida sarcoma cells. Tritiated thymidine was injected 30 minutes prior to MMC injection.

LEGENDS TO PHOTOS

Photo. 1. ~ 4. The effects of MMC on solid Yoshida sarcoma cells. Tritiated thymidine was injected 30 minutes prior to sacrifice. Photo. 1. The A zone at 6 hours after administration of 200 μg/kg of MMC. Photo. 2. The B zone at 6 hours after administration of 200 μg/kg of MMC. Photo. 3. The A zone in the control. Photo. 4. The B zone in the control.

Photo. 5. ~ 8. The effects of MMC on solid Yoshida sarcoma cells. Tritiated thymidine was injected 30 minutes prior to MMC injection. Photo. 5. The A zone at 6 hours after administration of 200 μg/kg of MMC. Photo. 6. The B zone at 6 hours after administration of 200 μg/kg of MMC. Photo. 7. The A zone in the control. Photo. 8. The B zone in the control.

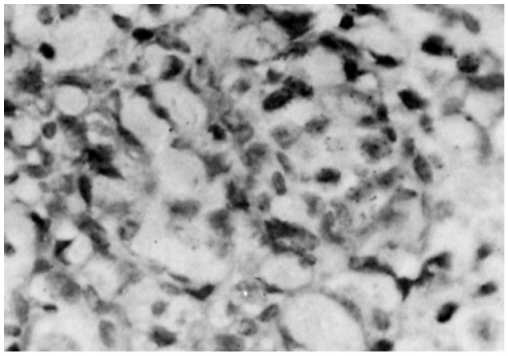

FIG. 1

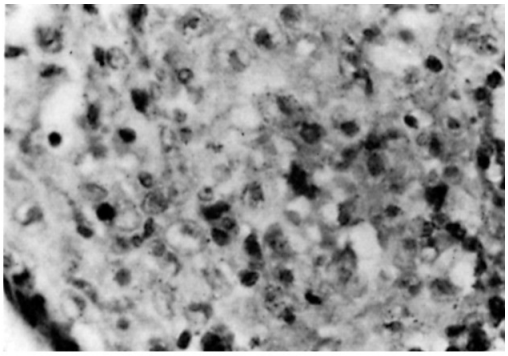

FIG. 2

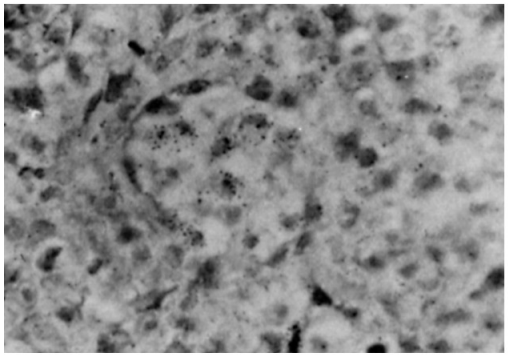

FIG. 3

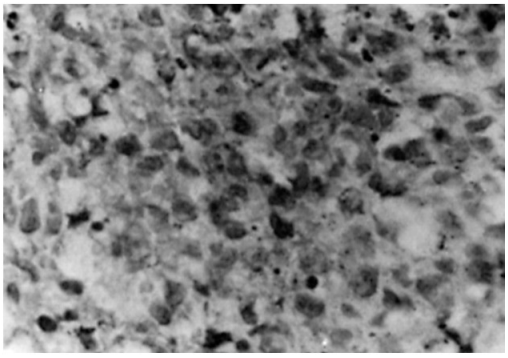

FIG. 4

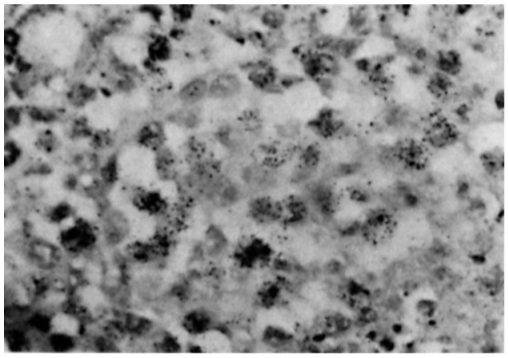

FIG. 5

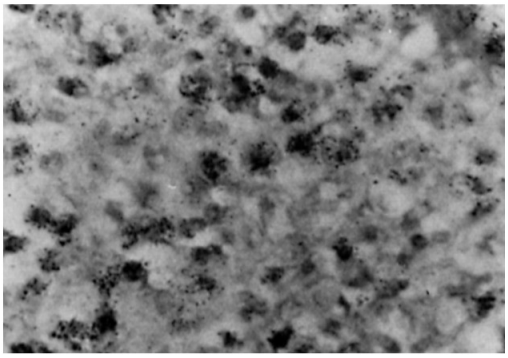

FIG. 6

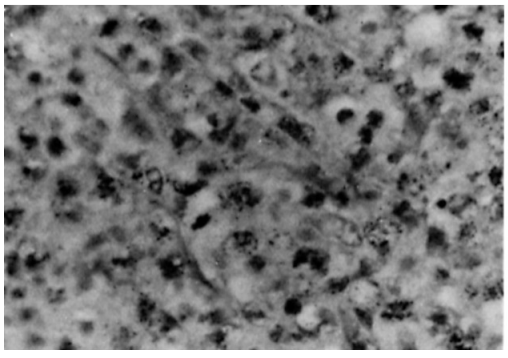

FIG. 7

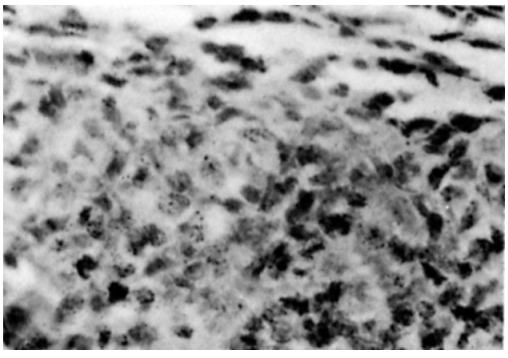

FIG. 8