EFFECT OF CYCLOHEXIMIDE ON THE CELL CYCLE OF THE CRYPTS OF THE SMALL INTESTINE OF THE RAT

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ABSTRACT

A single injection of 1.5 mg/kg of cycloheximide induces a complete disappearance of mitotic activity in rat intestinal crypts within 1.5–2 hr. No significant necrosis of crypt cells is observed even though this phenomenon is accompanied by a marked decrease in uptake of labeled precursors into protein and DNA. Mitoses reappear 6 hr after injection and recovery then follows a cyclic pattern over a period equivalent to one cell cycle, thereby reflecting at least a partial synchronization of cell division. Concurrent use of colchicine, an agent known to induce metaphase arrest, has demonstrated that cycloheximide, while having no apparent effect on cells already in division, prevents the entrance of new cells into visible mitosis. Analysis of the cell cycle suggests that one block initiated by cycloheximide occurs in G_2, presumably as the result of an interference with the formation of protein(s) required for the normal progression of cells from this phase of the cycle into mitosis.

INTRODUCTION

The discovery during the past several years of compounds with effects on macromolecular synthesis in intact cells permits the study of patterns of cellular reaction to interference with selected synthesis of important cellular compounds. As part of an intensive study of the response of certain cells, in vivo, to these agents, we first selected cycloheximide (Actidione), an antibiotic produced by *Streptomyces griseus* (42). This compound is known to inhibit protein synthesis in a variety of cells (4, 11, 12, 26, 49) and appears to do so by interfering with the transfer of amino acids from amino acyl–soluble RNA to the site of peptide bond synthesis on the ribosome (16, 35, 41).

Preliminary experiments with this antibiotic demonstrated that it is capable of inducing a profound effect on normal cell division in many tissues of the intact rat. In order to analyze this phenomenon in greater detail, we have studied the small intestine, an organ which provides a substantial population of continuously dividing cells. The results of this investigation indicate that cycloheximide induces a rapid and complete disappearance of all mitotic figures in the crypts of the small intestine without inducing obvious cell death. The evidence further suggests that cycloheximide initiates a complete arrest in the early G_2 phase of the cell cycle. However, the antimitotic effect of this antibiotic is fully reversible, with recovery following a cyclic pattern over a period equivalent to one cell cycle.

MATERIALS AND METHODS

In all experiments, male white Wistar rats (Carworth Farms) weighing 160–175 g were injected between 8 and 9 a.m., after a 12- to 15-hr period of fasting. Cycloheximide (The Upjohn Co., Kalamazoo, Mich.) in a concentration of 1 mg/ml, freshly prepared in 0.9% NaCl, was injected intraperitoneally. In some experiments, 0.15 mg of colchicine (Eli Lilly & Co., Pittsburgh, Pa.), suspended in 1.5 ml of saline, was injected subcutaneously. For incorporation studies,

the following isotopes (New England Nuclear Corp., Boston, Mass.) were injected intraperitoneally: L-leucine-1-^{14}C (20 mc/mmole; 25 μc/ml), thymidine-2-^{14}C (30 mc/mmole; 10 μc/ml) and uridine-^{14}C (30 mc/mmole; 10 μc/ml). Control rats received saline in corresponding volumes and by similar routes.

The animals were sacrificed by decapitation and a 15-cm segment of the small intestine was removed, beginning at the duodeno-pyloric junction. From this segment four samples, including Peyer's patches when available, were fixed in Stieve's solution for histological examination. Sections of paraffin-embedded tissue were stained with hematoxylin and eosin and the Feulgen method for DNA. The number of mitoses in ten adjacent high-power fields was counted in each of the four sections of intestine, and the mitotic activity then expressed as number of mitoses per high-power field (hpf).

INCORPORATION STUDIES: Groups of two or three animals were injected with cycloheximide or saline at zero time and with 5 μc of leucine-^{14}C, 7 μc of thymidine-^{14}C, or 7 μc of uridine-^{14}C after 10 min or 100 min. Following a 20-min period of incorporation, the animals were sacrificed and a 15-cm segment of small intestine corresponding to that used for histological examination was removed. This segment was rapidly washed free of its contents with ice-cold saline and then homogenized in cold 5% trichloroacetic acid (TCA) with a glass pestle. The precipitate, containing protein, RNA, and DNA, was washed two times with 5% TCA containing either nonradioactive leucine (5 mg), thymidine (1 mg), or uridine (1 mg) as carrier. Subsequently, it was washed once with 95% ethanol–10% acetate, once with absolute ethanol, twice with ethanol-ether (3:1) at 60°, and twice with ethyl ether. After the ethanol–K acetate wash, the precipitate was extracted twice with 10% TCA at 90° for 10 min to remove nucleic acids when leucine was used as the labeled precursor. 5 mg of the dried powder were dissolved in hyamine and a toluene scintillation-counting mixture and counted in a Packard liquid scintillation spectrometer at an efficiency of 65%. The results are here expressed as counts per minute (cpm) per milligram precipitate. This is justifiable since the RNA and DNA concentrations in the precipitate, as measured by the Logan et al. (28) modification of the Schmidt and Thannhauser method (34), are the same in control and experimental samples. The initial TCA extract (acid-soluble extract) was brought to a constant volume, and an aliquot of this was mixed with Bray's solution (6) and counted in each instance.

RESULTS

Inhibition of Cell Division by Cycloheximide

Preliminary experiments utilizing dosages ranging from 3.0 to 4.5 mg/kg provided the first evidence of the antimitotic effect of cycloheximide. Although these dosages were well tolerated by the majority of males, they were highly toxic to females. However, 1.5 mg/kg (equivalent to 0.25 mg for the weight range of the animals used), though nonlethal to both sexes, was sufficient to bring about a complete disappearance of mitotic activity in the crypts of the small intestine.

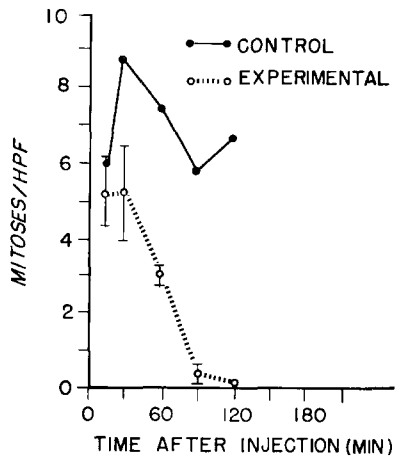

FIGURE 1 Mitosis in the intestinal crypts of the rat during the 1st 2 hr after the administration of a single injection of 0.25 mg of cycloheximide. Each point in the experimental groups represents the mean of two animals. Vertical bars represent ranges.

To follow the evolution of this phenomenon more closely, we determined the mitotic activity in sections of small intestine taken from one control and two experimental animals 15, 30, 60, 90, and 120 min after injection of 0.25 mg cycloheximide per animal. As can be seen from Fig. 1, there is a progressive decline in the number of cells undergoing division, culminating in their complete disappearance within 90–120 min after injection. It is also important to note that this inhibitory effect was not associated with any significant necrosis in the crypts (Fig. 2).

To determine whether cycloheximide similarly interfered with cell division in other tissues as well, we examined also its effect on regenerating liver. Since the results of our experiments, in agreement with those of other investigators (7, 9, 10, 22, 40), have shown that the peak of mitotic activity occurs 28 hr after partial hepatectomy, two animals were injected with saline and two with cycloheximide 26 hr postoperatively and then sacrificed 2 hr later. As in the small intestine, numerous dividing cells were seen in the controls, while neither mitosis nor

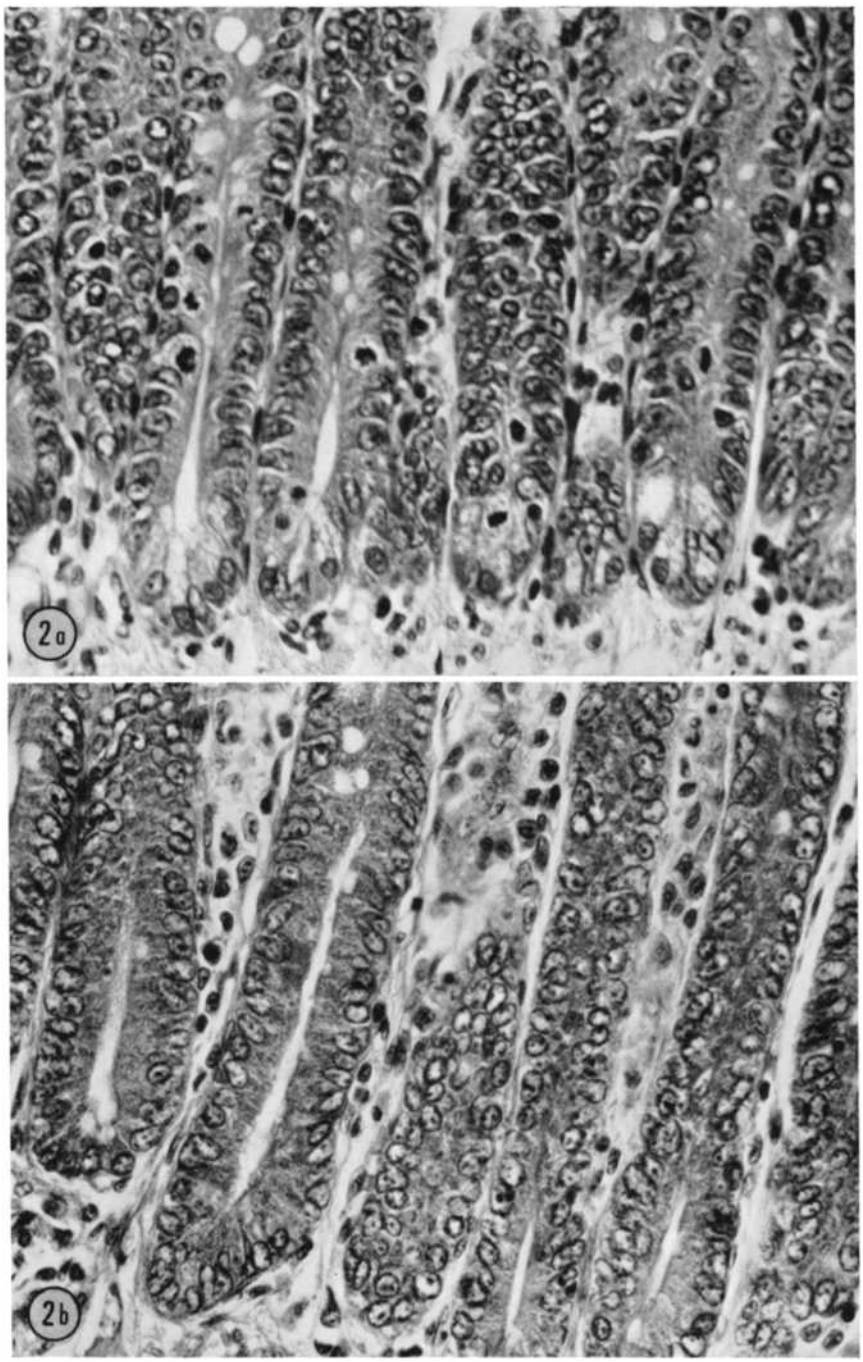

FIGURE 2 *a*, Crypts from a control animal, illustrating normal mitotic activity occurring in this area. *b*, Crypts from an animal treated with 0.25 mg of cycloheximide 2 hr previously, illustrating absence of mitosis and of necrosis. Hematoxylin and eosin. × 450.

cell death was observed in the experimental animals.

Analysis of the Disappearance of Mitotic Figures in Intestinal Crypts

These findings suggest at least two possible effects of cycloheximide: it may be selectively lethal to dividing cells or, alternatively, it may prevent the progression of additional cells into the mitotic phase of the cell cycle without having any direct effect upon those cells already in division. To test these hypotheses, we administered cycloheximide to animals prior to or following the administration of colchicine, an agent known to induce metaphase arrest. If cycloheximide blocks the cell cycle at some stage prior to the onset of mitotis, all cells beyond the site of the block would complete division while cells in a stage of the cycle preceding the block would be interrupted. If this is so, animals treated first with colchicine and then cycloheximide should show an initial increase in the number of cells arrested in metaphase, followed by a plateau. In contrast, if cycloheximide selectively destroys cells in mitosis, there should be a marked decrease in the number of cells arrested in metaphase.

21 rats were divided into three groups and then treated according to the schedule indicated in the legend accompanying Fig. 3. Animals receiving colchicine plus saline showed a linear increase, with time, in the number of cells arrested in metaphase, in agreement with the results of Stevens Hooper (36). Rats which were injected with saline at zero time and cycloheximide 0.5 hr later again demonstrated a complete disappearance of dividing cells. As expected, however, in those animals given colchicine at zero time and cycloheximide 0.5 hr later, the degree of metaphase arrest showed an initial increase over control levels and then remained essentially the same. This supports the hypothesis that, once cycloheximide is introduced into the system, progression into visible mitosis is prevented. Furthermore, the fact that the number of cells arrested in metaphase remained constant, rather than showing a rapid decrease, makes unlikely the alternative possibility that cycloheximide selectively destroys cells undergoing division. This interpretation includes the assumption that colchicine does not afford protection against the effect of cycloheximide.

Further support for the conclusion concerning the site of action of cycloheximide is derived from

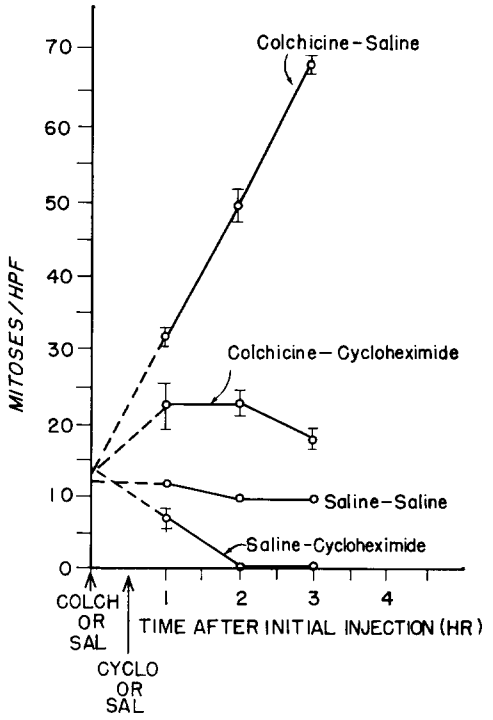

FIGURE 3 Effect of pretreatment with colchicine followed by exposure to cycloheximide on the number of mitoses in the crypts of the small intestine of the rat. In each of three groups, one animal was injected with 1.5 ml of saline, subcutaneously at zero time, and with 0.25 ml of saline, intraperitoneally 0.5 hr later; two were injected with 1.50 ml of saline, subcutaneously at zero time, and with 0.25 mg of cycloheximide, intraperitoneally 0.5 hr later; two were injected with 0.15 mg of colchicine, subcutaneously at zero time, and with 0.25 mg of cycloheximide, intraperitoneally 0.5 hr later; two were injected with 0.15 mg of colchicine, subcutaneously at zero time, and with 0.25 ml of saline, intraperitoneally 0.5 hr later. Groups I, II, and III were then sacrificed 1, 2, and 3 hr, respectively, after the initial injection. Each point represents the mean of two animals, except in the saline-saline-injected group in which only one animal was used at each time interval. Vertical bars represent ranges. Arrows on the abscissa indicate times of injections of saline (*sal*), colchicine (*colch*), or cycloheximide (*cyclo*).

experiments in which the animals were pretreated with cycloheximide for 2 hr. If cycloheximide does block the normal progression of the cell cycle, no cells should be entering mitosis 1.5–2 hr after its administration and, if colchicine is injected at the end of this period, virtually no cells should be observed in metaphase arrest. Seven rats were

treated according to the schedule in Table I. In those animals which received saline and colchicine, there was approximately a threefold increase in the number of mitoses over control levels. However, those animals receiving cycloheximide and saline or cycloheximide and colchicine all showed essentially no mitotic activity. The results of the experiment further support the hypothesis that cycloheximide, while having no effect on cells already in division, prevents additional cells from progressing into visible mitosis, if it is assumed that cycloheximide does not interfere with the action of colchicine.

TABLE I

*Schedule of Injections Used in Pretreatment with Cycloheximide Followed by Colchicine**

Zero time	2 hr
0.25 ml saline ip	1.5 ml saline sc (1)
0.25 mg cycloheximide ip	0.15 mg colchicine sc (2)
0.25 ml saline ip	0.15 mg colchicine sc (2)
0.25 mg cycloheximide ip	1.50 ml saline sc (2)

* Cycloheximide, 0.25 mg, injected intraperitoneally at zero time. Colchicine, 0.15 mg, injected subcutaneously 2 hr after cycloheximide. All animals sacrificed 3 hr after initial injection.

Abbreviations used: ip (intraperitoneal), sc (subcutaneous). Number of animals in parentheses.

Incorporation Studies

It is well established that cycloheximide inhibits protein synthesis in a variety of intact cells. Since it has been reported that cycloheximide may also inhibit synthesis of DNA and/or of RNA in various systems to different degrees, it became important to observe the response patterns of protein, DNA, and RNA syntheses of the intestine under the conditions used in this study. A single dose of 0.25 mg cycloheximide was injected intraperitoneally at zero time and leucine-^{14}C, thymidine-^{14}C, or uridine-^{14}C were injected 20 min prior to sacrifice. Table II shows the effects of cycloheximide treatment on the incorporation of these precursors into protein, DNA, and RNA of the small intestine.

The uptake of leucine-^{14}C into protein decreased abruptly to 3% of the control values within 0.5 hr after the injection of cycloheximide and remained at essentially this same level over the entire test

TABLE II

*Effect of Cycloheximide on the Incorporation of Precursors into Protein, DNA, and RNA by the Small Intestine of the Rat**

Time after injection of cycloheximide	Macromolecule	Animal	Incorporation	Acid-soluble fraction	Corrected values‡	Inhibition
hr			*cpm/mg*	*cpm/0.5 ml*		%
0.5	Protein	Control (2)	670 ± 123	554 ± 159	1.25 ± 0.13	—
		Treated (2)	18 ± 15	395 ± 142	0.04 ± 0.03	97
	DNA	Control (3)	450 ± 30	1154 ± 55	0.39 ± 0.03	—
		Treated (3)	73 ± 26	1211 ± 76	0.06 ± 0.02	85
	RNA	Control (3)	18 ± 6	717 ± 394	0.02 ± 0.02	—
		Treated (3)	25 ± 6	930 ± 47	0.03 ± 0.01	0
2.0	Protein	Control (2)	735 ± 67	385 ± 23	1.91 ± 0.06	—
		Treated (2)	187 ± 19	1645 ± 49	0.12 ± 0.01	94
	DNA	Control (3)	344 ± 161	768 ± 241	0.44 ± 0.07	—
		Treated (3)	145 ± 32	1159 ± 61	0.12 ± 0.03	70
	RNA	Control (3)	27 ± 6	1399 ± 478	0.02 ± 0.01	—
		Treated (3)	39 ± 6	1255 ± 334	0.03 ± 0.01	0

* Cycloheximide: 0.25 mg per rat injected at zero time. Isotopes were injected intraperitoneally 20 min prior to sacrifice. Number of animals in parentheses.

‡ Corrected for variation in acid-soluble radioactivity:

$$\frac{\text{Specific activity of protein, DNA, or RNA}}{\text{Radioactivity per unit volume acid soluble fraction}}.$$

period. DNA synthesis was 15% of control values at 0.5 hr, but rose to 30% by 2 hr. Cycloheximide had no apparent effect on the incorporation of uridine-^{14}C into RNA at either time interval. These results suggest that the block in DNA synthesis may be released earlier than the block imposed on the synthesis of protein. However, in all measurements of incorporation of a radioactive precursor, the level of radioactivity in a product is, in part, a function of the specific activity of the immediate precursor. As is evident in Table II, the uptake of each injected precursor by the tissue, as indicated by the levels of acid-soluble activities, does not appear to be influenced negatively by the injection of cycloheximide. The acid-soluble radioactivities are essentially the same in the treated and control animals at 0.5 hr, and are higher with thymidine or leucine at 2 hr.

Recovery of Mitotic Activity

For the determination of the duration of its antimitotic effect, cycloheximide was given at zero time and one control and two experimental animals were killed at 4, 6, 7, 8, and then every 2 hr over a 24-hr period. Fig. 4, in which the degree of mitotic activity in the inductive and postinductive periods is plotted, shows that there is essentially no mitotic activity under 6 hr after injection. At 6 hr, there is a sudden burst of mitotic activity which reaches a peak 1 hr later. From this point, there is a gradual but progressive decline in the number of mitoses until 16 hr after injection. Subsequently, the number of dividing cells again increases and, after reaching a peak 4 hr later, returns to control levels. Statistical analysis of these values revealed that the mean number of mitoses per HpF in the controls over the entire test period was 6.5 ± 1.1. In the experimental animals, at 7 and 16 hr, the mean number of mitoses was 14.7 ± 0.8 and 1.2 ± 0 per HpF, respectively, with $p \ll .001$ at each of these two points. Thus, the deviations from the control values at both 7 hr and 16 hr following cycloheximide are highly significant statistically. These results demonstrate that cycloheximide instituted a transient block of cell division which was followed by complete recovery within 24 hr. They also suggest that cycloheximide has induced some degree of cell synchrony, since the number of mitoses early in the recovery phase is considerably greater than that in the control animals. At no time was there any significant degree of necrosis in the crypts.

However, it is conceivable that, as a consequence of the inhibition of protein synthesis, there might be a decrease in cell size with a resultant increase in the number of cells in a unit area (i.e., high-power field). Therefore, the degree of cell

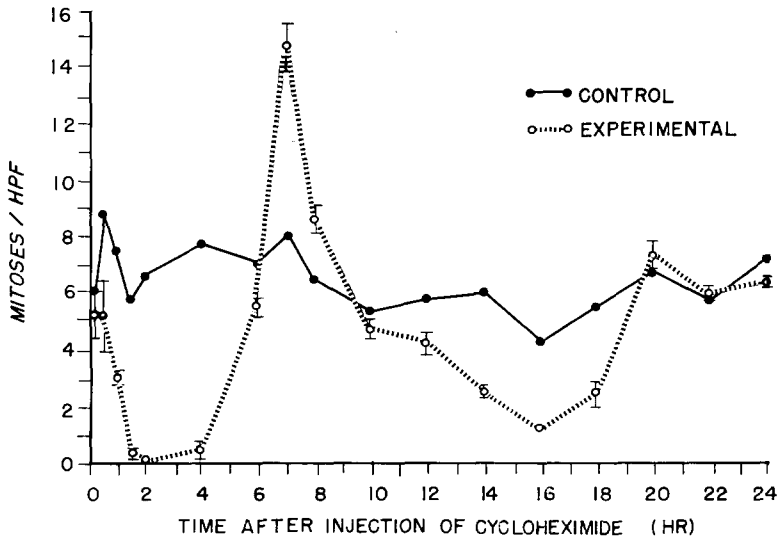

FIGURE 4 Effect of a single intraperitoneal injection of 0.25 mg of cycloheximide on mitotic activity in the intestinal crypts of the rat. Each point in the experimental groups represents the mean of two animals. Vertical bars represent ranges.

division was also determined in the above animals by counting the number of mitotic figures in ten crypts in each rat at each time interval. The results of this method produced a curve similar to that seen in Fig. 4, thus eliminating the possibility that these cyclic changes might merely reflect cyclic alterations in cell volume.

Effects of Multiple Injections of Cycloheximide

In order to determine the reaction of intestinal crypt cells to repeated episodes of cell cycle interruption by cycloheximide, we maintained a group of rats, on a regular diet and administered 0.25 mg cycloheximide every 24 hr for 4 days. Two saline-injected animals served as controls. Of the six rats treated with cycloheximide, two died between 8 and 23 hr after the 4th injection. The remaining four animals tolerated the procedure with no apparent ill effects and were killed 24 hr after the 4th injection. Histological examination of sections taken from the small intestine revealed a normal or perhaps slightly elevated mitotic activity in the crypts. Again, no necrosis was observed in these structures.

DISCUSSION

It is evident from the results of this study that cycloheximide is capable of virtually completely eliminating mitotic activity in the epithelial cells lining the small intestine without producing crypt cell necrosis. With the dose used, this effect lasts for about 6 hr and is followed by a return of mitotic activity to control levels.

An antimitotic effect of cycloheximide was first reported by Wilson (43) and subsequently confirmed in a number of additional studies utilizing pea and onion roots (5, 21, 24, 44, 46, 47). These investigations led to the conclusion that the antimitotic property of cycloheximide was due to its ability to prevent rupture of the nuclear membrane, thus interfering with the transformation to the metaphase configuration. If this were the mode of action of cycloheximide, the number of cells observed in mitosis should increase as a result of an accumulation of cells in prophase. The results of our study show that this is not the case in the intestinal epithelium of the rat. A single dose of 0.25 mg of cycloheximide led to the rapid and complete disappearance of dividing cells within 1.5–2 hr. This phenomenon appears to be due to the ability of cycloheximide to block the entrance of new cells into division while at the same time permitting cells already in mitosis to continue into postmitotic phases of the cell cycle. This, in effect, leads to an emptying of the M phase and accounts for the failure of the colchicine-cycloheximide–treated animals to sustain the linear increases in the number of mitoses observed with colchicine in control animals and for the complete absence of metaphase arrest when colchicine was introduced after pretreatment with cycloheximide

Since the detection of early prophase is extremely difficult in an in vivo mammalian system, it is conceivable that Wilson's explanation for the inhibitory effect of cycloheximide on dividing cells is a valid one. However, the time involved in the complete disappearance of mitotic figures argues strongly against this possibility. It appears more likely that the block initiated by cycloheximide occurs at some time prior to the onset of mitosis. Cairne et al. (8) have reported that the cell cycle in the crypts of the small intestine of the rat varies from 10.5–12 hr with S, G_2, M, and G_1 occupying 6.5–8, 1, 1, and 2 hr, respectively. The time required for the complete disappearance of mitotic activity in the crypts is essentially equal to the duration of G_2 plus M, or 2 hr. Therefore, if one considers G_1 to be the initial stage of the cycle and M the final, it can be tentatively concluded that the site of the last block induced by cycloheximide is located in G_2. Although the synthesis of DNA is also depressed, our data indicate that this block may be partially released prior to that imposed on protein synthesis. Furthermore, if there is a random distribution of cells over the whole course of the cell cycle, and if the lengths of the different phases as reported by Cairne et al. are correct, an S phase block should not result in a significant decrease in the mitotic rate until sometime after 2 hr following the administration of cycloheximide. The finding of a marked drop at 1 hr and complete disappearance at 2 hr makes it highly improbable that an S phase block is the last site of interruption in the cycle. Additional support for this conclusion is derived from the experiment involving regenerating liver. Since the bulk of DNA synthesis required for the first wave of mitosis has already been completed by 24 hr postoperatively (20), the effect of cycloheximide injected at 26 hr cannot be attributed to its interference with the formation of this nucleic acid. Instead, as in the small intestine, the ability to induce a complete disappearance of mitotic activity by 2 hr, without any indication

of a selective destruction of dividing cells, is compatible with a block in the postsynthetic or G_2 phase of the cycle.

It has been well established that during G_2 cells are actively engaged in the synthesis of RNA and protein, but not DNA (1, 2, 13, 23, 31, 33, 37). The results of our experiment indicate that cycloheximide profoundly inhibits protein synthesis but has no apparent effect on the incorporation of uridine into RNA, a finding similar to that reported in *Physarum polycephalum* (15). Therefore, the G_2 block precipitated by cycloheximide can be attributed tentatively to its interference with the synthesis of protein(s) required for the normal progression from G_2 into visible mitosis. This conclusion is consistent with reported observations in the slime mold, *Physarum polycephalum* (14, 15), and in Chinese hamster ovary cells in vitro (38). It also appears that the synthesis of this protein(s) takes place at a specific time in G_2. If progression from this phase of the cycle into mitosis were dependent on continuous protein synthesis, all cells residing in G_2 would be equally susceptible to the effects of cycloheximide and their advancement into division would be prevented. As a result, a complete disappearance of mitotic activity would be seen within 1 hr. The observation that 1.5–2 hr are required for this phenomenon to occur indicates that: (*a*) only a certain percentage of cells are affected, presumably those which have not, as yet, synthesized this essential protein(s); and (*b*) the formation of the protein(s) required for the movement of cells from G_2 into mitosis is restricted to early G_2. Consequently, the advancement into division of those cells which have already completed protein synthesis would be unimpaired while that of those cells which have not as yet done so would be impeded. This possibility might also explain Wilson's observations, in pea root meristems, that the administration of cycloheximide to cells in G_2 resulted in the splitting of the cell population into two parts; one part entered mitosis at about the normal time while the other was delayed (45).

By utilizing the values assigned to the various stages of the cell cycle and by assuming a total generation time of 12 hr, we can offer a possible explanation for the apparent cyclic pattern in which recovery occurred. The first significant rise in mitotic activity was seen 6 hr after injection. Since 1 hr is required for the passage of cells from G_2 into M, the block initiated by cycloheximide was about 5 hr in duration. Subsequently, the number of mitoses increased rapidly, reaching a peak at the 7-hr interval. That this event occurred 2 hr after the release of the block provides additional evidence in support of a G_2 block. It appears that, during the course of this blockage, an increased proportion of the cell population, as compared to the control tissue, accumulates in G_2. Once released, these cells progress into division, thus accounting for the significant but temporary overshooting of control levels. In addition, the magnitude of this rebound is highly suggestive of some degree of cell synchrony. The gradual decline that occurred between the 7th- and 16th-hr intervals is thought to indicate the passage of the remainder of the cell population through division. Since this involved a 9-hr period, it can be assumed that these cells were formerly located in the S phase of the cycle. However, since 10 hr would actually be required for the complete traversal of all cells from S through M, the low point of this descending portion of the curve should have occurred at the 17-hr interval. The second rise in mitotic activity is presumed to mark the second reappearance in division of those cells originally blocked in G_2. Inasmuch as this event requires a span of 14 hr from the time the block is released, the number of dividing cells should reach a second maximal level at the 19-hr interval. The results of our experiments are consistent with these expectations if allowance is made for the fact that our observations were made at 16 and 20 hr instead of the calculated intervals. Recovery, then, follows a cyclic pattern which, when measured with respect to the time lapse between the two peaks of mitotic activity (7 and 19 hr), is equivalent to one cell cycle.

It appears that the effects of cycloheximide, in interfering with the dynamics of the cell cycle without inducing cell death, are not unique to the intestinal epithelium of the rat, since a similar depletion of mitotic figures can be seen in the regenerating liver following partial hepatectomy. These results indicate that cycloheximide, in appropriate dosage, may be a useful agent in the analysis of the relation of cell division to physiological activity of a variety of cell types in the intact animal.

Examination of the biochemical events occurring during the induction of mitotic inhibition revealed a marked reduction in the incorporation of precursors into both protein and DNA; RNA synthesis, however, appears to be unaffected. These

observations are in agreement with those reported by Bennett and his coworkers in cultured human epidermoid carcinoma and mouse adenocarcinoma (3). Estensen and Baserga (17), on the other hand, have demonstrated a 90% inhibition of RNA synthesis in mouse intestine within 2 hr after the administration of 10 mg of cycloheximide per animal. The apparent conflict in results may be due to species difference or to the larger dose of cycloheximide utilized by Estensen and Baserga.

Phillips et al. (32) report severe karyorrhexis in the intestinal crypts of rats following the administration of hydroxyurea, a specific inhibitor of DNA synthesis (48). Similarly, puromycin, a potent inhibitor of protein and nucleic acid synthesis (18, 19, 25, 27, 30), has also been reported to induce severe intestinal damage within hours in both rats (29) and mice (17). A unique feature of cycloheximide, on the other hand, is its apparent ability to disrupt several aspects of the cell cycle in the crypts of the small intestine without inducing subsequent necrosis. Examination of these structures at frequent intervals both prior to and following the inhibition of mitosis, induced by a single dose of 0.25 mg, failed to reveal any significant degree of pyknosis or other criteria commonly associated with cell death. Even animals given this same dose daily for 4 days failed to reveal crypt damage when examined 24 hr after the fourth injection. From these observations, it would appear that factors other than those which merely interfere with the formation of protein or DNA or even with cell division must be responsible for cell death. It is possible that the duration or mechanism by which this occurs is more important than the inhibition itself.

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