ACTION OF ACTINOMYCIN ON PROLIFERATING EPITHELIUM OF THE DUODENAL CRYPTS IN MICE

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After a single injection of actinomycin into mice, mitosis in the epithelium of the duodenal crypts took place at the normal intensity for the first 6 h. The number of mitoses then gradually fell to reach a minimum after 22-24 h, rising after 28-30 h to reach the normal level after 50-52 h. The character of change in the curve of proliferative activity during the first 10-12 h after injection of actinomycin indicates that all periods of interphase in a large proportion of cells are not sensitive to the action of actinomycin.

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Investigation of the cells of several tissues has shown that disturbance of RNA synthesis by actinomycin during early interphase inhibits mitotic division, while its disturbance at the end of interphase does not prevent the normal course of the mitotic cycle [1, 2, 4]. The first part of interphase is described as sensitive to the action of actinomycin, while the second part is actinomycin insensitive.

The object of this investigation was to determine sensitivity of various periods of interphase in cells of the duodenal crypts of mice to the action of actinomycin.

EXPERIMENTAL METHOD

Noninbred male albino mice (136) weighing 20±2 g were used in the investigation. Actinomycin was injected intraperitoneally in a dose of 0.25 μg/g at 10 A. M. or 10 P. M. The animals were sacrificed during the first 36 h after the single injection of actinomycin at intervals of 4 h for 12 h and intervals of 6 h for the next 3 days (2-4 mice at each time).

Mitoses were counted in 20 crypts of each animal. The number of mitoses was expressed as a percentage of the total number of cells in the crypts.

To determine the duration and character of the mitotic cycle the mice received thymidine-H^3 (specific activity 1 Ci/mmole) by intraperitoneal injection in a dose of 0.6 μCi/g (the times of injection are given during the description of the experimental results). The animals were sacrificed (2 mice at each time) at intervals of 2 h for 16 h. The percentage of labeled mitoses was calculated at the various times after injection of thymidine-H^3 (100 mitoses were counted for each animal) and curves showing the change in number of labeled mitoses were plotted [6]. The labeling index (the number of labeled cells expressed as a percentage of the total number of cells in the crypts) was determined for some of the mice 1-2 h after injection of thymidine-H^3.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that during the first 6 h mitosis in the epithelium of the crypts took place at about normal intensity (about 3%). The number of mitoses thereafter gradually fell to reach a minimum (0.1-0.2%) after 22-24 h. A gradual increase in the number of mitoses began 28-30 h after injection of actinomycin, and normal proliferative activity was restored after 50-52 h.

Determination of the length of the mitotic cycle from the curve expressing changes in the percentage of labeled mitoses in the control animals (Fig. 2a) showed that its duration in the epithelial cells of the duodenal crypts of mice is about 10 h (this is close to published figures [3, 5]). Of this period about 1.5 h

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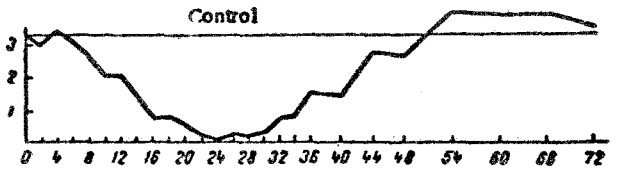

Fig. 1. Changes in level of mitotic activity of epithelium of duodenal crypts of mice during 3 days after a single injection of actinomycin. Abscissa, time (in h) after injection of actinomycin; ordinate, number of mitoses (in %)

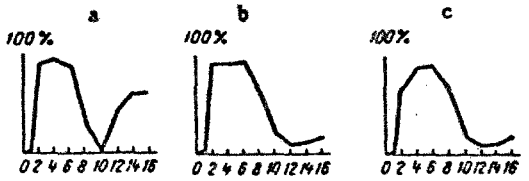

Fig. 2. Changes in percentage of labeled mitoses after a single injection of thymidine-H^3 in control animals (a), after a single injection of thymidine-H^3 given 4 h after injection of actinomycin (b), and 28 h after injection of actinomycin (c). Abscissa, time (in h) after injection of thymidine-H^3; ordinate, number of labeled mitoses (in %).

is occupied by the postsynthetic period G_2 and about 6 h by the period of DNA synthesis (the S period). The second wave of labeled mitoses which was well-marked in the control animals can be seen on the curve.

When thymidine-H^3 was injected 4 h after actinomycin, the duration of stages of the mitotic cycle such as stages G_2 and S, in the time interval when the number of mitoses was gradually falling (see the corresponding segment of the curve in Fig. 1), remained close to normal (Fig. 2b). The second wave of labeled mitoses was absent.

When thymidine-H^3 was injected 28 h after actinomycin, at a time when a gradual increase in the number of mitoses was taking place, the duration of periods G_2 and S were not significantly different from normal (Fig. 2c). The second wave of labeled mitoses was absent in this case also.

At each particular moment the epithelial cells of the crypts are evidently at various stages of interphase (about 3% are actually dividing). After a single injection of actinomycin (and also under normal conditions) the first cells to begin mitosis were those which were at the end of interphase (in the G_2 period) at the time of the injection, followed in turn by cells which were at the end, in the middle, and at the beginning of the S period, while the last to start mitosis, i.e., 7-10 h later, were cells at the beginning of interphase or in the presynthetic G_1 phase at the time of injection.

Since during the first 6 h after injection of actinomycin the intensity of mitosis was about normal, it can be concluded that the late part of interphase (G_2 and a large part of the S period) in the whole cell population of the crypt epithelium is insensitive to disturbance of RNA synthesis by actinomycin. The gradual decrease in the number of mitoses in the time interval between 6 A. M. and 10 A. M. indicates that the early portion of interphase in part of the cell population is sensitive to the action of actinomycin.

Despite the gradual decrease in number of mitoses, the mitotic activity remained reasonably high (2%) 10-11 h after injection of actinomycin. Consequently, a large proportion of the cell population was insensitive to the action of actinomycin throughout interphase, including in its early part.

The fall in number of mitoses continuing after 10-11 h demonstrated that disturbance of RNA synthesis caused by a single injection of actinomycin affected the normal completion of the mitotic cycle in cells of the second generation also.

Determination of the labeling index 5-6 h after injection of actinomycin showed a slight decrease (from 45% under normal conditions to 36%). Hence it can be concluded that some of the cells were held up in transition from stage G_1 to the stage of DNA synthesis. Delay in periods G_1 and G_2 (in the G_2 period of cells of the second generation) is perhaps indicated by absence of the second wave of labeled mitoses which we noted during the gradual decrease in the level of proliferative activity.

In the interval between 30 h and 54 h after injection of actinomycin the number of dividing cells gradually increased. This suggests that within this time interval not only the cells which had divided at the end of the preceding mitotic cycle started to undergo division (as happens normally), but also cells which had

divided much earlier and had been delayed in a certain stage of interphase. This hypothesis is confirmed by the character of the curve of labeled mitoses. We observed that the second wave of labeled mitoses was absent in this case also, despite the increase in the absolute number of mitoses which had occurred (see Fig. 2c and the corresponding segment of the curve in Fig. 1). A possible explanation of all these findings is commencing division of many cells which had divided not one mitotic cycle previously (because they were not labeled), but much earlier, and had been delayed in their passage through interphase. They were possibly held up in the stage G_2, after completing DNA synthesis.

The labeling index 29-30 h after injection of actinomycin was 25%, indicating that delay in passage of the cells from stage G_1 to the stage of DNA synthesis persisted and was perhaps even intensified.

The response of the epithelium of the duodenal crypts of mice to disturbance of RNA synthesis after a single injection of actinomycin can thus be described as follows. In part of the cell population all periods of interphase, including its early portion, are insensitive to the action of actinomycin, while in other cells the early portion of interphase is sensitive to the action of actinomycin. Disturbance of RNA synthesis by actinomycin affects normal completion of the mitotic cycle in cells of the 2nd generation also. Cells not starting mitosis at the appointed times are delayed at certain stages of interphase (stages G_1 or G_2). This long delay (more than 20 h) accounts for the gradual decrease in number of mitoses. The subsequent gradual increase in number of mitoses (restoration of the normal level of proliferative activity) is the result of commencing mitosis by cells delayed in the earlier periods after injection of actinomycin.

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