EFFECT OF ACTINOMYCIN C ON THE REGENERATION OF THE LIVER IN MICE

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Regeneration in the liver of mammals is characterized by peculiar changes in the metabolism of nucleic acids having a direct connection with the process of cell division in the regenerating organ [2-5, 7, 8, 11, 12]. In recent times the characteristics of nucleic acid metabolism have been investigated by the use of inhibitors, among which is actinomycin. The action of actinomycin (C or D) results in a depression of the synthesis of RNA which is dependent on DNA. It has been shown that actinomycin inhibits the synthesis of all kinds of RNA in the regenerating liver of rats [9]. We have not met with any work in which the action of actinomycin on the process of liver regeneration has been examined cytologically.

The aim of the present work was to study the action of actinomycin C on the regeneration of the liver in mice and, in particular, on the process of cell division, the synthesis of DNA and the hypertrophy of the cells.

EXPERIMENTAL METHODS

White, male mice weighing 23 ± 2 g were used in the experiments. In the first series of experiments, after partial hepatectomy (by the standard method of Higgins and Anderson), the mice were injected intra-abdominally three times (directly after the operation, after 24 h and after two days) with 2 μg actinomycin C in 0.5 ml physiological solution (small dose). In the second series of experiments after partial hepatectomy, the mice were injected similarly with 10 μg actinomycin C (large dose). The greater number of animals were killed by decapitation three days after the operation (always within 8-9 h) but the smaller number were killed one day or two days after the operation. Each of these animals received a dose of two or four μg, or 10 or 20 μg actinomycin C respectively.

Pieces of tissue were fixed in Carnoy's solution and embedded in paraffin wax. Sections having a thickness of 7 μ were cut and stained with Weigert's hematoxylin. From each animal the number of cells covering a standard field of view (diaphragm 7 × 7 mm, objective 90 ×, ocular 7 ×) were counted (total from 30 fields) and this served as an indirect determination of their sizes; the mitotic index (per 1000 on a count of 6000 cells) and the number of binucleate cells (percent on the total number of cells on a count of 600 cells) were determined. In addition, measurements were made of the nuclei (on 200 nuclei) in some of the animals. The regenerating livers of mice which had not been injected with actinomycin C and the livers of nonoperated mice were used as controls in both series of experiments.

In an additional, third series of experiments, ten nonoperated mice were injected three times (once per day) with 10 μg actinomycin C (total 30 μg) and killed by decapitation after three days.

EXPERIMENTAL RESULTS

The results of the first and second series of experiments are given in the table as arithmetical averages of all the measurements carried out. The duration of the experiments was three days.

Changes in the Regenerating Liver of Mice Injected with Actinomycin C

Experimental conditions	Number of animals	Weight of regenerated liver, mg	Mitotic index, ‰	Number of cells in field of view	Number of binucleate cells, %
Nonoperated mice	6	–	–	28.00	16.10
Regeneration without injection of actinomycin C	8	806	11.90	20.90	7.50
Regeneration, actinomycin C (small dose)	16	773	0.47	19.24	13.05
Regeneration, actinomycin C (large dose), group "a"	3	780	0.00	18.20	24.70
Regeneration, actinomycin C (large dose), group "b"	6	480	0.00	26.60	25.20

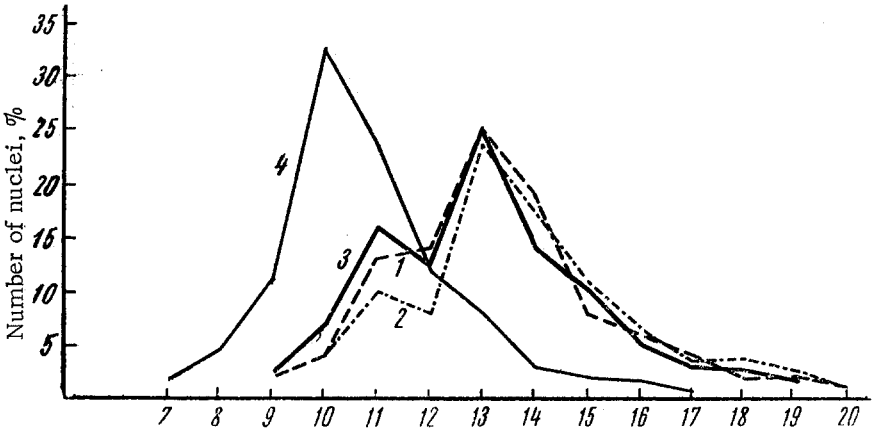
It may be seen from the table that, in regeneration associated with the injection of small doses of actinomycin C, the weight of the regenerated liver, three days after the operation, was almost unchanged when compared with the control (regeneration without actinomycin). Besides this, the mitotic division of the cells was very strongly inhibited. The dimensions of the cells, which we judged from the number in the field of view, increased to approximately the same extent as in normal regeneration. In other words, during regeneration associated with small doses of actinomycin C, hypertrophy of the cell elements was similar to that in normal regeneration. The number of binucleate cells observed during regeneration associated with small doses of actinomycin C was rather less than in the normal liver. In these instances the number of binucleate cells was not halved as was characteristic for the three day normal regeneration.

According to the results of the second series of experiments (large dose of actinomycin C) the animals fell into two groups ("a" and "b"). In the first of these groups (1/3 of the animals) the weight of the regenerating liver was scarcely different from that of the controls, that is, regeneration took place in spite of the large dose of actinomycin C. In the second group (2/3 of the animals) regeneration was insignificant or was absent altogether. Mitosis in the regenerating liver was absent in both the first and second groups. The dimensions of the cells in the first group of animals increased to approximately the same extent as those in the normal regenerating liver.

Thus, in regeneration associated with large doses of actinomycin C the usual hypertrophy of the cell elements of the liver over three days was observed. In the second group of animals hypertrophy of the liver cells was absent. The number of binucleate cells in both groups of animals increased (24-25% compared with 16.1% in normal livers and with 7.5% in normal regenerating livers over the same period of time).

Data from earlier periods of regeneration are not given in the table. It should be pointed out that, two days after the operation in the first series of experiments, a considerable inhibition of mitosis was observed 60.2 ‰ in comparison with 18‰ in the control) and in the second series it was absent altogether. The day after the operation mitosis was absent in both series of experiments as it was in the control. This goes to show that during regeneration associated with injection of actinomycin C, the peak of mitotic activity is not shifted to an earlier period, but that inhibition (partial or complete) of mitotic division was noted throughout the whole of the first three days of regeneration.

The additional, third series of experiments, in which nonoperated mice were injected with 30 μg actinomycin C, was carried out in order to discover if the number of binucleate cells increased during regeneration associated with the injection of large doses of actinomycin C. In the liver of such mice the number of binucleate cells almost doubled in three days; in the normal liver there were 16.1‰ and after the injection of 30 μg of actinomycin C there were 30.1‰ The results of measuring the nuclei of normal and regenerating livers (both with and without actinomycin C) are given in the figure. In the regenerating liver associated with the injection of small and large doses of actinomycin C (first group of animals in the second series of experiments) the nuclei increased in size as in the regenerating liver of mice which had not been injected. In the second group of animals in the same series of experiments there was no change in the size of the nuclei.



Changes in the dimensions of the nuclei in regenerating liver of mice. 1) Regeneration associated with the injection of small doses of actinomycin C; 2) regeneration associated with the injection of large doses of actinomycin C; (group "a"); 3) regeneration without injection of actinomycin C (normal); 4) liver of non-operated mice.

The data which we have obtained show that mitotic cell division in the regenerating liver of mice is strongly or entirely inhibited by actinomycin C. At the same time, actinomycin serves as a stimulating factor in amitotic cell division, both in normal and regenerating livers. An increase in the number of binucleate cells in the complete absence of mitosis (second and third series of experiments) gave us grounds for sharing the point of view of those authors who have affirmed that binucleate cells in the liver are the result of amitotic nuclear division. The absence of a reduction in the number of binucleate cells in the first and second series of experiments, in which mitosis was strongly or completely inhibited, verifies experimentally that such cells, evidently, divide almost always amitotically.

As is well known, the sizes of the cell nuclei in any one tissue are correlated with the amount of DNA which they contain and an increase in the sizes of the nuclei indicates, of course, indirectly, that the synthesis of DNA is going on within them [1, 6, 10]. An increase in the size of the cell nuclei in the regenerating liver, associated with small doses of actinomycin C, indicates indirectly that DNA is being synthesized in the nuclei. Thus, it may be presumed that the synthesis of DNA by the nuclei of liver cells is, probably, far from being the only condition for the onset of mitotic division in these cells. Actually, in the regeneration of liver associated with the injection of actinomycin C and when DNA is being produced, mitotic cell division is strongly or completely inhibited. For the onset of mitotic cell division it is evident that, not only is the synthesis of DNA necessary but also the undisturbed synthesis of RNA (or the nonobstruction of the molecules of DNA).

On the basis of our data it may also be thought that the regeneration of liver in mice, during the first three days under conditions of strong or complete inhibition of mitotic cell division by actinomycin C, goes on almost freely (in the sense of weight increase of the organ). This may be accounted for by the normally occurring hypertrophy of the cells, by the increase in the size of the nuclei and by the stimulation of amitotic cell division by actinomycin.

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