THE EFFECT OF SARCOLYSIN ON THE 24-HOUR PERIODICITY OF MITOSES IN SOME ALBINO RAT TISSUES

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Studies on the effects of various experimental factors which may either stimulate or inhibit the mitotic activity in tissues are often conducted without taking into consideration the 24-h mitotic periodicity. This may often lead to incorrect conclusions, because the changes in the numbers of mitoses observed may be due not to factors under investigation but to the natural 24-h mitotic periodicity.

It must also be considered that changes in the mitotic activity following various experimental manipulations may vary depending on the time of day when these experimental factors are applied. Sufficient formation on this subject is available [2, 10, 13-15].

Sarcolysin is an agent which interferes with the processes of nucleoprotein metabolism and DNA synthesis during interkinesis, and which produces significant changes in the mitotic activity of tissues [1, 8, 9]. However the effect of this preparation on the mitotic activity of different tissues has not been studied in sufficient detail.

The purpose of this work has been to study the effect of sarcolysin on the mitotic activity of different tissues taking into account the normal 24-h mitotic periodicity, which had been studied earlier [2-7, 11, 12]. It was also important to determine the significance of the time of day when sarcolysin was introduced into the animals, in order to evaluate the subsequent changes in the level of the mitotic activity and the nature of the 24-h periodicity of cell divisions.

EXPERIMENTAL METHODS

Male albino rats weighing 160-170 grams were used in our experiments. The animals were divided into three groups: the first group consisted of control animals which were not affected in any way; the second group consisted of animals which received sarcolysin at 6 a.m., i.e., at the time of day when maximum mitotic activity is noted in most tissues; the third group consisted of animals which received sarcolysin at 10 p. m., i.e., during minimal mitotic activity in many organs.

The preparation was introduced intrapertioneally in the amount of 12 mg/kg.

First animals in each group were killed 4 h after the injection, i.e., at 10 a. m., for the second group and at 2 a. m., for the third group. Subsequently the rats were killed at 4-h intervals during 24 h (7 experimental and 7 control rats at each time interval). Organs were fixed in Carnoy's fluid. Mitotic coefficients were estimated as promilles to the total number of cells. The data obtained were subjected to statistical analysis using the Fisher-Student test.

EXPERIMENTAL RESULTS

A distinct 24-h periodicity of cell divisions with a maximal number of mitoses between 6 and 10 a. m., and the minimal number between 6 and 10 p. m., was noted in the corneal epithelium of control animals. In animals

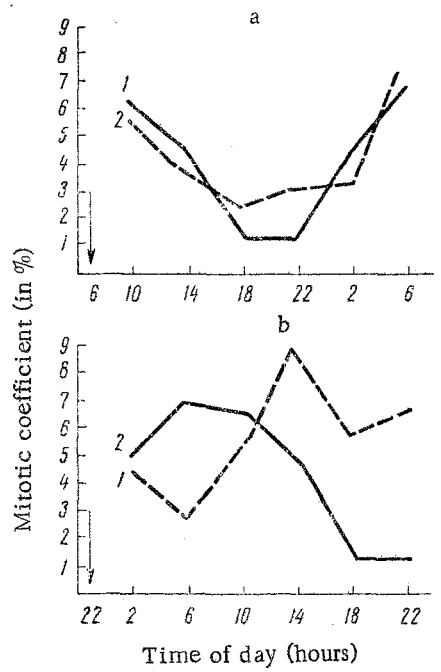

Fig. 1. Variation of the 24-h mitotic periodicity in the esophageal epithelium of rats with a morning (a) and an evening (b) injection of sarcolysin. 1) Control rats; 2) experimental rats.

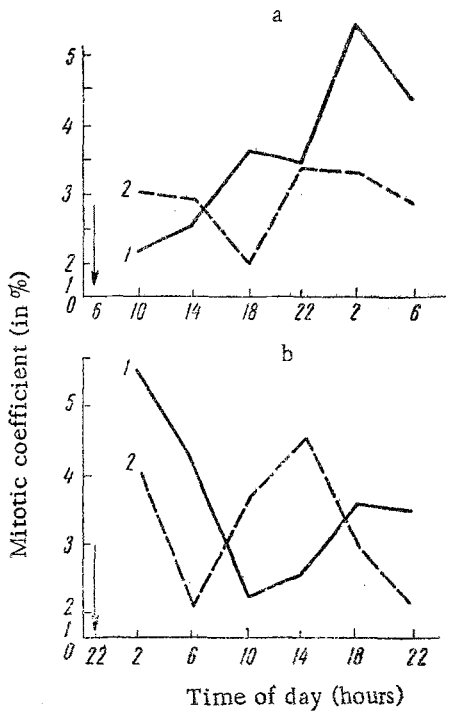

Fig. 2. Variation of the 24-h mitotic periodicity of the epithelium of the stomach fundus of rats with a morning (a) and an evening (b) injection of sarcolysin. Legend as in Fig. 1.

of the second and third groups there appeared to be no significant changes in the mitotic activity and the nature of the 24-h periodicity of cell divisions.

Similar results were obtained with adrenals. An exception was a significant lowering of the number of mitoses in the glomerular zone in the animals of the third group at 10 p. m., i.e., after 24-h following the injection.

In the epithelium of the lower third of the esophagus of control animals there was a distinct 24-h periodicity of mitosis (Fig. 1).

Twelve to sixteen hours after the morning injection of sarcolysin the number of mitoses in the experimental rats was found to be higher than in the control ones ($P = 0.05$ and 0.01 respectively).

In the esophageal epithelium of animals in the third group the number of cell divisions at 6 a. m., was higher in the control animals and lower in the experimental ones. As a result of this there arose significant differences in the numbers of mitoses between the groups which were compared ($P = 0.004$). Beginning at 10 a. m., the mitotic activity in the esophageal epithelium of rats in the third group rose and subsequently remained at a high level, while in control rats it became lower in accordance with the normal 24-h mitotic periodicity. Thus, an inversion of the 24-h mitotic periodicity was seen in the esophageal epithelium following an evening injection of sarcolysin.

The increase in the number of mitoses noted in the esophageal epithelium of rats of the third group 12 h following the sarcolysin injection is difficult to explain. Apparently it was not related to a slowing down of any stage of mitosis, because in the animals of all three groups there were not found any significant differences in the relative numbers of the different stages of mitosis.

In the livers of control rats there was also noted a 24-h mitotic periodicity with a maximum at 6 a. m., and a minimum during the rest of the day. In livers of animals of the second group there was no usual increase of cell divisions at 6 a. m. Thus, in this tissue as well as in the glomerular zone of the adrenal gland, the inhibitory effect of sarcolysin bec me revealed 24 h after its injection.

In livers of rats of the third group there was a 24-h periodicity of mitosis similar to that in the control animals. At the same time at 2, 6 and 10 a. m., i.e., 4-12 h after injection, the number of mitoses in the experimental animals was higher than that in the control ones ($P = 0.012$).

In the epithelium of the stomach fundus of control animals the maximal mitotic activity was noted between 2 and 6 a. m. and the minimal between 10 a. m. and 2 p. m. (Fig. 2). After a morning injection of sarcolysin the 24-h differences in the numbers of mitoses were less pronounced and statistically insignificant because of considerable individual differences. After an evening injection there was noted a double peaked curve in the 24-h mitotic periodicity with maximum numbers of cell divisions at 2 a. m. and 2 p. m., and minimum numbers at 6 a. m., and 6-10 p. m. Changes in the mitotic activity in the two groups were seen to be

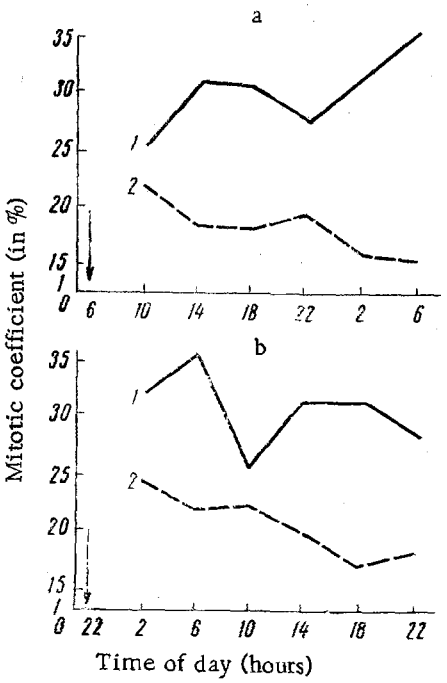

Fig. 3. Variation of the 24-h mitotic periodicity of the epithelium of the crypts of the small intestine of rats with a morning (a) and an evening (b) injection of sarcolysin. Legend as in Fig. 1.

inverted: at the times when in the control animals there was a decrease in the number of mitoses, it increased in the experimental animals and vice versa.

In the epithelium of the duodenal crypts of the control rats a significant increase in the number of mitoses was noted at 6 a. m., but in animals of the second and the third experimental groups there was neither a significant increase nor a decrease in the number of mitoses at any time interval. At the same time the total number of cell divisions became decreased. The mean 24-h mitotic activity in control animals constituted 30.2% in animals of the second group – 20% ($P = 0.0001$) and in the third group 18.8% ($P = 0.0001$).

A similar picture was noted during the study of the small intestine (Fig. 3). The 24-h mitotic periodicity was completely disrupted following either a morning or an evening introduction of sarcolysin. The decrease in the number of mitoses began as early as 4 h following injection; later the decrease became pronounced to a progressively greater degree, so that towards the end of the 24-h period the decrease in the number of mitoses became statistically significant. The mean 24-h mitotic activity in the control rats constituted 30.4%, in animals of the second group 18.4% ($P = 0.0001$), and in those of the third group 20.7% ($P = 0.001$).

In the epithelium of the large intestine of control animals there was no definite 24-h mitotic periodicity. In the epithelium of the large intestine of rats in the second group there were considerably fewer mitoses at almost all the time intervals. The mean 24-h mitotic activity in the control animals constituted 14.7% and in those of the second group 9.7% ($P = 0.001$).

In animals of the third group as early as 4 h following the sarcolysin injection there was some decrease in the number of mitoses, but towards 10 a. m. (12 h after injection) the number of mitoses rose sharply and at this time exceeded the number of mitoses in the control rats. During the second half of the day in the control and the experimental animals the number of mitoses decreased gradually but in the experimental animals it was consistently lower (10.8%) than in the control animals ($P = 0.008$).

Thus, sarcolysin produces different changes in the mitotic activity and in the 24 h periodicity of cell divisions in different rat tissues, and the nature of these changes depends on the time of day when the substance is injected.

The following hypothesis presents itself in explanation of this phenomenon. It is known that the interkinetic period of cells which are preparing for mitosis may be divided into several phases: post-mitotic phase, phase of DNA synthesis and pre-mitotic phase. Each of these is characterized by its own biochemical peculiarities and the duration of each of these phases apparently varies in different tissues. It may be supposed that the development of these phases as well as of the mitotic phase is subject to daily variations. If a factor under study has a specific effect on any definite phase, then, naturally, the effect of this factor on the initiation of cell division will be more effective if at the time of its action most cells of a given tissue are in that particular phase of preparation for mitosis. It may be expected that sarcolysin, which is an alkylizing compound, has a preferential effect on the DNA synthesizing phase of the process of preparation for mitosis.

The above data also show that a correct evaluation of action of factors which either stimulate or inhibit cell division may be made only when 24-h variations in the number of mitoses are known. If such differences are ignored, quite contrary results may be obtained in similarly conducted experiments, depending on what time of day the animals are killed. This can be seen from the curves of 24-h variations of the mitotic activity in the esophageal epithelium of rats in the first and third groups (Fig. 1). When control and experimental animals were killed at 6 a. m., it appeared that sarcolysin delayed cell divisions; when the animals were killed at 10 a. m., it might have been supposed that sarcolysin had no effect on the mitotic activity in the esophagus; and, finally, when the animals were killed during the period between 2 and 10 p. m., it would have appeared the sarcolysin stimulates cell division.

It is obvious that the method of killing control and experimental animals always at the same hour of day in order to avoid daily variations in the number of mitoses does not always preclude an arrival at incorrect conclusions.

SUMMARY

A study was made of the effect produced by sarcolysin (12 mg/kg) on the mitotic activity of various tissues in albino rats following its administration in the morning (6 a. m.) and in the evening (10 p. m.) hours. No significant changes of the average 24-h mitotic activity or any disturbance of the 24-h mitotic periodicity were revealed in the corneal epithelium, fascicular and reticular zones of adrenal cortex and in the medulla; this had no relation to the timing of the drug administration.

Irrespective of the period marking the injection of the preparation the epithelium of duodenal crypts and, especially, that of the jejunum presented a progressive decline in the number of mitoses and an upset diurnal periodicity of the latter. The glomerular area of the adrenal, 24 h after the evening administration, and that of the liver, following the morning injection, showed an authentic reduction in the number of cellular divisions occurring at time-periods coinciding with the maximum mitotic activity in control animals. But in 4-12 h after the morning injection the liver was noted to become a scene of an appreciably increased mitotic activity with the diurnal periodicity of the latter continuing its normal course. The same phenomenon is observed to occur in the esophageal epithelium 12-16 h after a morning injection of sarcolysin. At the same time an inverted regular diurnal periodicity of mitosis was recorded in the epithelium of the esophagus and of the large intestine, after evening injections, and in that of the gastric fundus, regardless of the time marking the drug administration.

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