DURATION OF MITOSIS IN THE CORNEAL EPITHELIUM OF FASTING ANIMALS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 57, No. 1,
pp. 95-98, January, 1964
Original article submitted January 16, 1963

We have previously shown that the level of mitotic activity in the corneal epithelium of fasting animals, distinguished by minimal energy expenditure per unit weight, was maintained for a long time within normal limits[3]. However, despite the maintenance of the number of dividing cells at a constant level during a definite period of fasting, the actual rate of mitosis could be varied. The object of the present investigation was to shed light on this problem, which is of importance when determining the proliferative power of epithelial cells in conditions unfavorable to the organism.

EXPERIMENTAL METHOD

Experiments were conducted on sexually mature male albino rats weighing about 200 g, deprived of food for 2, 3, and 7 days. The animals received water ad lib. Control animals were given a normal diet. Experimental and control animals were sacrificed simultaneously. Altogether 60 rats were used.

Mitoses were counted in total preparations of the corneal epithelium of the eye stained with hematoxylin. The whole cornea was examined. The mitotic index was taken to be the number of dividing cells in 100 fields of vision, corresponding to 1 mm^2 area of the corneal epithelium.

To determine the duration of mitosis a method was used which was based on the delay of the passage of the cells from a stage of interkinesis to the prophase stage by means of x-rays followed by counting the rate of disappearance of the individual phases of mitosis [6-9]. The rats received whole-body irradiation with x-rays in a dose of 400 R. The investigations of I. A. Utkin [8] showed that this dose does not affect the actual duration of mitosis.

In each of the three series of experiments 4 groups of animals (with 5 rats in each group) were used: 1) irradiated and on a normal diet; 2) irradiated and fasting; 3) unirradiated and on a normal diet; 4) unirradiated and fasting.

TABLE 1. Disappearance of Mitoses in Normally Fed Rats and Rats Fasted for 2 Days

Group of animals	Mean number of mitoses					
	Prophase	Metaphase	Anaphase	Telophase	Late telophase	Mitotic index
Control unirradiated	23	49	11	20	18	121
Control irradiated	0.2	1	2	3	5	11
Fasting unirradiated	28	52	9	15	17	121
Fasting irradiated	0	1.2	1.3	2	3.6	8

EXPERIMENTAL RESULTS

In the first series of experiments mitoses were counted in rats sacrificed 53 min after the beginning of irradiation (Table 1). The results demonstrated that after fasting for 48 h the number of mitoses in the experimental and

control animals was the same (the mitotic index in both was equal to 121). The rate of disappearance of all the phases of mitosis in the fasting and control rats was also equal. It is clear from the results given in Table 1 that the number of mitoses in the control unirradiated animals in the stage of late telophase was 18, compared with only 5 mitoses in the irradiated rats (P = 0.004).

A similar picture of disappearance of mitoses was also observed in the fasting rats: in the unirradiated animals in the stage of late telophase there were 17 mitoses compared with 3.6 in the irradiated animals (P = 0.001).

TABLE 2. Disappearance of Mitsoses in Normally Fed Rats and Rats Fasted for 3 Days

Group of animals	Criterion determined	Pro-phase	Meta-phase	Ana-phase	Telo-phase	Late telophase	Mitotic index
Control unirradiated	Mean number of mitoses	10	60	12	30	18	130
Control irradiated	Mean number of mitoses	0	2	1	5	13	21
P		0.000	0.000	0.000	0.007	0.347	
Fasting unirradiated	Mean number of mitoses	10	87	19	31	25	172
Fasting irradiated	Mean number of mitoses	0	2	3	7	14	26
P		0.000	0.000	0.000	0.000	0.013	

TABLE 3. Disappearance of Mitoses in Normally Fed Rats and Rats Fasted for 7 Days

Group of animals	Criterion determined	Pro-phase	Meta-phase	Ana-phase	Telo-phase	Late telophase	Mitotic index
Control unirradiated	Mean number of mitoses	17	34	6	16	22	95
Control irradiated	Mean number of mitoses	0	2	3	10	12	27
P		0.000	0.000	0.01		0.011	
Fasting unirradiated	Mean number of mitoses	5	21	5	10	10	51
Fasting irradiated	Mean number of mitoses	0	1	1	1	3	6
P		0.000	0.000	0.01	0.000	0.01	

Having found no difference in either the number of dividing cells or the duration of mitosis in the experimental and control rats, we lengthened the period of fasting to 3 days. The animals in this series of experiments were sacrificed 47 min after the beginning of irradiation. The difference between the number of mitoses in the normally fed and the fasting rats was not statistically significant (Table 2). At the moment of sacrifice, prophase, metaphase, anaphase, and telophase had all taken place in the normally fed and the fasting animals. The difference between the number of telophases (30 in the control unirradiated and 5 in the control irradiated rats) was statistically significant (P = 0.007). The fact that the difference between the number of mitoses in the stage of late telophase (18 in the control unirradiated and 13 in the control irradiated rats; P = 0.347) is not statistically significant indicates that 47 min after irradiation the very last stage of mitosis had not yet been completed. Since 85% of all mitoses had been successfully completed in a period of 47 min, the duration of mitosis was 55 min.

A similar comparison may be made for the fasting unirradiated and irradiated rats. As Table 2 shows, 47 min after irradiation the stage of telophase was practically complete. The number of late telophases was approximately half the number of late telophases in the unirradiated fasting animals. Hence, 47 min after irradiation 92% of the phases of mitosis were completed, so that the duration of mitosis was 51 min.

Hence, the mean rate of mitosis in the rats after fasting for 3 days was very close to that in the control animals. The slight difference lay within the limits of experimental error. The total number of dividing cells in the fasting rats was slightly larger than in the controls, but, as mentioned above, this difference was not statistically significant. It is interesting that after fasting for 2 and 3 days, not only mitosis, but also interkinesis followed a normal course, for otherwise, with a change in the period of interkinesis but no change in the duration of mitosis, the total number of dividing cells would either increase or decrease [8].

In the next series of experiments an attempt was made to determine the rate of mitosis after a longer period of fasting (7 days), when a definite decrease in the number of dividing cells had occurred (Table 3). The rats were sacrificied 45 min after irradiation.

Forty five minutes after irradiation 77% of the total number of mitoses were completed in the control irradiated rats. Consequently, the duration of mitosis was 58 min. In the fasting animals 45 min after the beginning of irradiation all the phases of mitosis were practically completed. It may be concluded from the results of this experiment that the duration of mitosis in the rats fasted for 7 days did not exceed 45 min, ie., that it was increased roughly by 21% over that in the control animals. Consequently, there is reason to suppose that the general effect of a diminution of mitotic activity (95 mitoses in the control and 57 mitoses in the experimental animals: $P = 0.01$) was partly due to an increase in the duration of mitosis. However, the main cause of the diminution of mitotic activity in the fasting rats was an increase in the duration of interkinesis, for even an increase of 21% in the duration of mitoses could not bring about a reduction in the number of dividing cells of almost one-half. The mean duration of mitosis, on the basis of three measurements in the control animals, was 55 ± 1 min, which agrees with I. A. Utkin's figure [8].

No visible differences in the shape and size of the nuclei of the corneal epithelial cells were found in the control and fasting rats. These facts are in harmony with the findings described by A. V. Rumyantsev and A. K. Belousova [1, 6], who showed that during fasting the mass of the cell nucleus and its ability to synthesize nucleoproteins are maintained, or even increased. This maintenance or increase in the synthetic activity of the nucleus are evidently very important in the preservation of the viability of the cell, by ensuring that mitosis is possible for a long period of time. Furthermore, the maintenance of the nucleoprotein balance of the nucleus probably enables the rapid restoration of mitotic activity to take place when considerably depressed as a result of fasting, after the administration of components such as lipids and carbohydrates, mainly concerned with the supply of energy [4].

The prolonged maintenance of the normal rate of mitosis in the corneal epithelium of fasting rats, in conjunction with the well marked diurnal periodicity of mitotic activity [5], demonstrates the preservation of the ability of the cornea to regenerate and to carry on its normal function.

SUMMARY

X-ray irradiation was used to study the duration of mitosis in the corneal epithelium of albino rats after 2, 3, and 7 days of starvation. As established, after 2 and 3 days of starvation there was no difference in the rate or number of mitoses in starving and control animals. It was concluded from these data that the period of interkinesis was the same in experimental and control animals. In rats starving for 7 days the rate of mitosis proved to be somewhat increased; however, the main cause of reduction of the number of dividing cells was the increase of the length of interkinesis.

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