DYNAMICS OF THE MITOTIC ACTIVITY IN CORNEAL EPITHELIUM OF WHITE MICE DURING FASTING

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The study of the interrelationship between metabolic processes and cellular multiplication is at the present time one of the important ways of exposing the mechanisms regulating cellular division. From this point of view, the study of the cellular multiplication process during the action on the organism of factors which significantly change or disturb the dynamics of metabolic processes becomes expeditious.

By utilizing the generally known fact [4] concering the lowering of the metabolic level during fasting, the authors decided to utilize this occurrence as a methodical procedure for the study of the dependence of cellular multiplication on the intensity of metabolic processes in the organism.

A number of authors [5, 6, 7] who have investigated the mitotic activity of skin epidermis, intestinal epithelium and of the liver from white mice and rats, have noticed a lowering in the number of mitoses during fasting. However, the majority of the investigators in these experiments did not encounter an especially high sensitivity of the cells toward various changes in the surrounding medium, among which were changes connected with the experiment itself. The latter could basically influence the mitotic regimen. This circumstance can be ascertained only when a sufficiently large number of control animals is introduced into the experiment. It is necessary to note that up to the present time the dynamics of mitotic activity during the fasting process, have not been sufficiently studied. Authors of a large number of similar experiments did not concern themselves with the role of metabolism in the concurrently occurring changes. The mitotic activity of such a convenient study object as the corneal epithelium of mammals has actually been subjected to an investigation during fasting. Only partial data [1,2] have been obtained on several animals.

Previously completed investigation [3] concerning the dynamics of mitotic activity in corneal epithelium of fasting young rats has shown that mitotic activity became lowered only on the third day and was dependent on the degree of the animal's exhaustion. A supposition was expressed that changes in mitotic activity during fasting were connected to the degree of exhaustion of the organism's energy resources.

Taking into account that mice have a higher basal metabolism than rats, the author decided to investigate the dynamics of mitotic activity in corneal epithelium during fasting of these animals.

In order to clarify some links in the mechanism responsible for the change in mitotic activity during fasting, an attempt was made to compensate the energy losses of the fasting animals by giving them carbohydrates.

EXPERIMENTAL METHODS

The experiments were conducted on 105 sexually mature male white mice with an average weight of 20 g. For the experiment the animals were held for 5-10 days under standard conditions with 5 animals per group. For each experimental group there was a corresponding control group.

In the first series, the experiment animals, as opposed to the controls, were not given feed and were given only water.

In the second series, besides the control group and the fasting group, there was a group of mice receiving unlimited amounts of carbohydrates (20% solution of sugar and small pieces of sugar or 40% glucose). The feeding and watering of the mice was conducted at the same time in all experiments. The animals were killed after 1, 2, 3, 4, or 6 days of fasting. Mitoses were counted in total histological preparations of the cornea, in its epithelial layer, with a consequent counting of the number of mitoses in 100 visual fields, corresponding to 1 mm^2 of its area.

EXPERIMENTAL RESULTS

Average number of mitotic indices of the first experimental series, according to each interval of complete fasting, are presented in Table 1.

As is evident from the data in Table 1, fasting for the period of one day did not produce a lowering of mitotic activity in the corneal epithelium of the experimental animals. In fasted mice during this fasting interval, there

TABLE 1. Mitotic Activity of Corneal Epithelium From Fasting White Mice

Control mice				Fasting mice			Remarks
duration of the experiment (in days)	number of mice	mitotic index	weight changes (in percent of the original)	No. of mice	mitotic index	wt. changes (percent of original)	
1	5	338	+3	5	409	—10	
2	5	305	Without change	5	49	—22	
	5	456	" "	5	36	—25	
3	5	463	+6	4	45	—28	Toward the end of the experiment one died
4	5	218	Without change	2	60	—34	During the experiment 3 mice perished
6	5	300	" "	2	41	—35	The same

was observed even an increase in the mitotic number (to be truthful, it was statistically insignificant), in a way similar to the observation in rats after two days of fasting [3]. The weight of experimental mice decreased by 10%.

Fasting of mice for the period of two days, which was accompanied by weight loss of 25%, led to a sharp lowering in the mitotic activity of the corneal epithelium (6-12-fold) in comparison with the control (P = 0.000). In animals fasted for three days, there was a 28% weight loss. Mitotic activity of experimental animals was 10 times lower than in the control group (P = 0.0000). From among five mice, one perished toward the end of the experiment. During fasting for four days only two out of five experimental animals survived. The weight of the fasted animals decreased by 34%. Mitotic activity of fasted mice appeared considerably lower than in fed mice (P = 0.0000). With a more prolonged fasting period (6 days), only 20% of the experimental mice survived (in this experiment 10 animals were fasted, 5 in each group). Weight loss of these mice comprised 35%, mitotic activity was decreased almost 8-fold (P = 0.02).

The sharp decrease of mitotic activity in the experimental mice of the first series, which was accompanied by a large weight loss during the fasting, could be explained first of all by an insufficiency of energy materials for the processes of cellular proliferation. A second series of experiments was conducted in order to clarify the above question; energy expenditures of the fasting mice were restored by feeding them sugar or glucose. Results of these experiments (mean mitotic indices and weight changes of the animals) are presented in Table 2.

From the data presented in Table 2 it is evident that in fasted mice the mitotic index after two days of the experiment was lowered by more than 6-fold, while at the same time in sugar-fed mice the number of mitoses in the corneal epithelium was not different from the controls (the difference was not statistically significant (P=0.845).

The fact that animals receiving sugar lost only 5% of weight when the fasted lost 20% deserves attention. Supplemental feeding with carbohydrates alone for four days (mice were given a 40% glucose solution) left the mitotic activity practically at the level characteristic for control animals (difference statistically not significant; P = 0.565).

At the same time, of the five fasted mice receiving only water, two were left alive; the mitotic activity was lowered 4-fold.

The data for mitotic activity are in agreement with the data for weight loss; mice receiving glucose lost 8.6%, mice receiving only water, 34% of weight.

TABLE 2. Mitotic Activity of Corneal Epithelium of Fasting and Carbohydrate-Fed Mice

Length of the fasting interval (in days)	Control			Full fasting			Sugar feeding		
	number of mice	mitotic index	weight change (in percent of initial mouse weight)	number of mice	mitotic index	weight change (in percent of initial mouse weight)	number of mice	mitotic index	weight change (in percent of initial weight)
2	5	358	Without change	5	54	20	10	347	5
4	5	220	" "	2	63	34	5	191	8.6
5	5	249	" "	5	All died		5	148	17

Extension of the glucose-only (40% solution) feeding period to five days (inclusive) resulted in pronounced lowering of the mitotic activity ($P = 0.004$). The weight of mice receiving glucose dropped by 17%.

The obtained data indicate that in mice mitotic activity was lowered earlier (on the second day) than in rats (on the third day).

This could be explained by the fact that in mice, which are distinguished by a high level of basal metabolism, during fasting there takes place rapid utilization of energy stores. In this case the compensation of energy stores occurs at the expense of protein destruction [4], and is evidently the final cause for the pronounced lowering of mitotic activity in the corneal epithelium of fasting animals. It should be noted, however, that even during the stage of pronounced exhaustion produced by the fasting, when the animals lost 25-35% of the initial weight, mitoses did not disappear completely.

Materials of the experiments where the fasting mice were fed carbohydrates gave basis for supposing, that during compensation of the organism's energy stores by carbohydrates alone, the mitotic activity of the corneal epithelium was supported at the normal level for four days of fasting inclusively. This is evidently explained by the presence of a sufficient amount of flexible materials in the organism which, while not being utilized for energy needs during fasting, make possible the processes of physiological regeneration at a level close to normal. However, after five days of fasting and in glucose-fed mice there occurred a pronounced lowering of mitotic activity. This lowering, evidently, occurs due to a disturbance, in the flexible-materials metabolism, produced by protein insufficiency.

SUMMARY

The dynamics of mitotic activity of corneal epithelium was studied in fasting white mice. The animals were deprived of food for a period of 1 to 6 days. A sharp decrease of mitotic activity, appearing from the second day of fasting, remained on the same level with more prolonged food deprivation as well. In mice which received only carbohydrates (sugar and glucose) the mitotic activity of the corneal epithelium remained normal up to 4 days inclusively; after the fifth day of experiment, however, the mitotic activity decreased. The loss of weight in mice given water only was four times greater then in those fed on carbohydrates.

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