MITOTIC ACTIVITY DURING MUSCULAR EXERTION

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The functional activity of the cells and the general functional activity of the body represent one of the factors which determine the daily pattern of cell division in the animal body [1,6,7]. The interrelation between cell division and the functional activity has attracted the attention of research workers for a long time. Changes in the mitotic activity connected with the general functional exertion of the whole body, however, have hardly been studied. Bullough [7] has shown that during intensive muscular exertion (running in a rotating drum) the mitotic activity decreases. And conversely: under conditions of drug-induced sleep the multiplication of cells becomes more intensive. The cause of these changes remains obscure.

In the present paper we studied changes in the mitotic activity during short-lasting muscular exertion and made attempts to establish the mechanism of these changes.

METHOD

The experiments were carried out on frogs and on three-month-old male white mice. The mitotic activity in the mice was assessed in the corneal epithelium. The intensity of the mitotic activity was assessed by the number of cells in a state of division within a standard area (1.65 mm^2), by the percentage proportion of various mitotic phases and by the phase coefficient. The findings were evaluated statistically by the method of Fisher and Student.

In the first series of experiments we studied changes in the mitotic activity during intensive muscular exertion. The intensive exertion of the mice consisted of swimming in a water bath at a temperature of 37°C. The swimming was continued for 45 min. During the week preceding the experiment the mice were subjected to preliminary daily training (five min). At various times after the swimming for 45 min, the mice were killed (after 45 min, one hour, $2\frac{1}{2}$ hours, four hours and six hours after the beginning of the exertion). In all experiments the mice were killed at the same time of the day. Animals kept under similar conditions but not subjected to muscular exertion served as control.

In the second series similar experiments were carried out on white mice after preliminary bilateral adrenalectomy. The bilateral adrenalectomy was carried out three-four days before the experiments.

In the third series of experiments (carried out jointly with Yu. B. Temper) we studied the influence exerted by the metabolic products of working muscles upon the cleavage of frogs' eggs. The hind legs of frogs were perfused with Ringer's solution through the aorta by the method of Trendelenburg. The perfusion was repeated several times with one and the same solution (1-1.5 ml). The perfusate was collected before (control) and after (experimental) stimulation of the muscles. The contraction of the muscles in various groups of experimental animals was induced either by direct stimulation or by stimulation of the nerves in the lumbosacral plexus. The stimulation was induced by induction current through an interrupter (30-40 stimuli per min). The contraction of the muscles was continued for 3-5 min and followed by a rest period. The periods of work and rest were repeated 3-4 times until the muscle became tired. Eggs of frogs (Rana nigromaculata, R. chensinensis) were submerged into the perfusate. The times of appearance and of completion of consecutive cleavage furrows were recorded under the microscope.

In the fourth series of experiments we studied the changes in the mitotic activity of corneal epithelium of mice after injection of perfusate from working muscles. The perfusate was administered by intraperitoneal injection (0.2-0.3 ml). The material was investigated 1-$1\frac{1}{2}$ hours after the injection.

RESULTS

The results of the experiments showed that during intensive muscular exertion the mitotic activity decreases in the corneal epithelium (Table 1).

TABLE 1. Changes in the Mitotic Activity in the Corneal Epithelium of Mice during Muscular Exertion

Group of mice	No. of animals	Time elapsed after beginning of experiment	No. of mitoses	Phase proportion, %				P
				prophase	metaphase	anaphase	telophase	
Control	5	—	134	18,4	30	6,6	45	0,001
Experimental	8	1 hr 10 min	38	11	18	4	67	
Control	6		86	12	33	8	47	
Experimental	6	2½ hr	21	60	8	14	18	0,001
Control	5	—	96	35	19	4	42	
Experimental	6	45 min	67	27	47	5	21	0,04
"	5	4 hr	125	25	18	6	51	0,08
Control	5	—	124	25	14	8	53	
Experimental	5	6 hr	104	36	14	7	43	0,1

Already within 45 min after the beginning of the exertion (i.e., immediately after completion of the exertion), a decrease in the mitotic activity could be observed in some animals. After one hour the number of cells in a state of division decreased almost to one quarter, and 2½ hours after the beginning of the exertion the mitotic activity was still on a very low level. Four hours after the exertion the mitotic activity was restored and even exceeded slightly the original level. Six hours after the beginning of the exertion the intensity of cell multiplication had reached its normal values. The decrease in the mitotic activity was accompanied by a gradual fall in the number of early mitotic phases. A new wave of mitoses appeared already within 2½ hours, when the level of mitotic activity was still very low (increase in the relative proportion of prophases). The shifts in the percentage proportion of the various mitotic phases warrant the assumption that the changes in the mitotic activity during muscular exertion are due to an inhibition preventing the cells from starting the process of division.

The character of changes in the mitotic activity during muscular exertion suggests that these changes are connected with an increased production of adrenaline. Adrenaline represents a powerful factor which prevents the cells from beginning the process of division. On the other hand, intensive muscular exertion is followed by a hypersecretion of adrenaline [8]. Taking into account these facts, we carried out the second series of experiments, in which we studied the changes in the mitotic activity during muscular exertion in mice subjected to bilateral adrenalectomy.

TABLE 2. Changes in the Mitotic Activity in the Corneal Epithelium of Adrenalectomized Mice during Muscular Exertion

Group of mice	No. of animals	Time elapsed after beginning of expt.	No. of mitoses	Phase proportion, %				P
				prophase	metaphase	anaphase	telophase	
Control	5	—	96	23	38	6	33	
Experimental	6	1 hr 10 min	118	5	26	5	64	0,1
Control	6	—	110	23	32	4	41	
Experimental	6	2 hr	11	38	0	0	62	0,001

The results of these experiments showed (Table 2) that one hour ten min after the beginning of the exertion no decrease in the mitotic activity occurred in the adrenalectomized animals. Only a relative fall in the number of prophases could be observed, a phenomenon usually observed in the beginning stages of the mitotic process. A marked inhibition of cell division could be observed in the adrenalectomized mice only after two hours.

Comparison of the mitotic activity in healthy and adrenalectomized mice, respectively, shows (Fig. 1) that in the absence of the adrenal glands the decrease in the number of cell divisions during muscular exertion is delayed.

In nonoperated mice the mitotic activity begins to decrease within 45 min and after one hour is already on a very low level. In adrenalectomized animals this decrease is marked only two hours after the beginning of the exertion. The initial inhibition of the mitotic activity during intensive exertion is apparently due to the secretion of adrenaline into the blood stream. The subsequent inhibition of mitosis is apparently induced by other factors. The findings of Heilbrunn and Wilson [10] warrant the assumption that these factors are the metabolic products of the working (tired) muscle. The above authors observed that sea water in which the working muscle had been kept inhibits cell division.

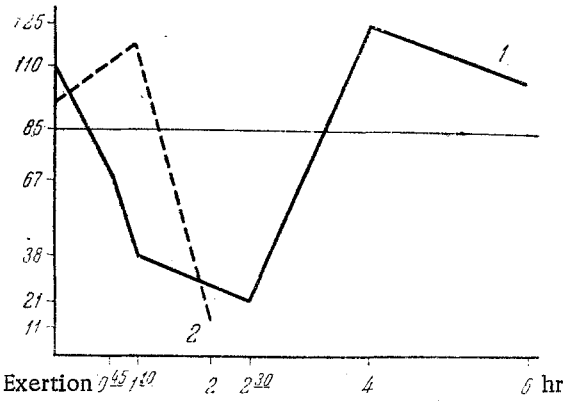

Fig. 1. Changes in the mitotic activity during muscular exertion in nonoperated (1) and adrenalectomized (2) mice. On the abscissa: the time (in hours); on the ordinate the number of mitoses were plotted.

In the third series of experiments we studied the rate of cleavage of frogs'eggs sumerged into the perfusion liquid which had been passing through the blood vessels in the hind legs of frogs. The control eggs were kept in a perfusate collected before the muscular contraction. The eggs of the experimental group were submerged into a perfusate collected after the muscular exertion.

The results of these experiments showed that certain substances develop in the working muscles which inhibit the cleavage of the egg cells (Fig. 2). The inhibition of the cleavage took place soon after submersion of the eggs into the perfusate and persisted throughout the 3-4 hours of observation.

In the majority of experiments the effect was more marked in the beginning of the observation and became weaker toward the end of the experiment. The development of six-seven cleavage furrows in the experimental eggs was on the whole delayed for 30-35 min compared

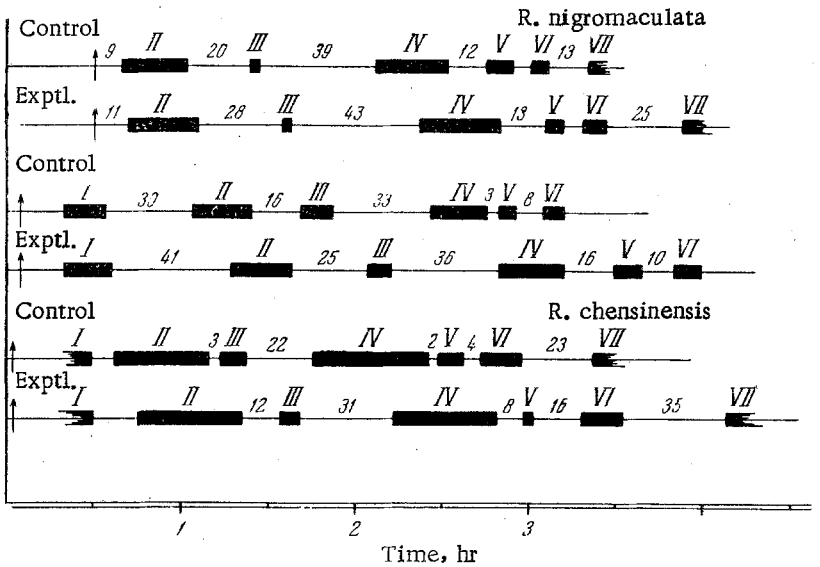

Fig. 2. The rate of cleavage of frogs'eggs. The thick parts of the lines illustrate the time of cleavage, the thin parts the stage of interkinesis. The Roman numbers indicate the consecutive cleavage furrows; the Arabic numbers show the duration of the interkinesis.

with the control eggs. The delay in the cleavage of the eggs was mainly due to a prolonged stage of interkinesis. The speed of division itself was on the whole unaffected and the deviations which could be observed in some cases did not exceed the range of possible error. The duration of the interkinesis was appreciably prolonged (in some cases by 10-12 min).

The effect of the metabolic products developing in the working muscles is of unspecific character. The perfusion liquid which had been passed through the blood vessels of frog muscles before and after contraction was injected into white mice of the control and the experimental group respectively. Injection of the perfusate from working muscles caused a decrease in the mitotic activity in the corneal epithelium (Table 3). This decrease was relatively small and not always sufficiently marked, but it occurred in all three groups of experiments performed by us. In two groups of similar experiments a decrease in the relative proportion of prophases could be observed.

TABLE 3. The Influence of Perfusate Which Had Been Passed through Working Muscles upon the Mitotic Activity in the Corneal Epithelium of Mice

Group of animals	No. of animals	No. of mitoses	Phase proportion, %				P
			pro-phase	meta-phase	ana-phase	telo-phase	
Control	5	136	24	38	4	34	<0,001
Experimental	5	82	18	38	5	39	
Control	5	176	26,5	31	4,5	38	
Experimental	5	101	17	38	4	40	0,01
Control	6	80	25	29	6	39	
Experimental	6	55	24	37	4	40	0,02

The results of our experiments thus warrant the assumption that the second factor responsible for the inhibition of cell division during intensive muscular exertion consists of the metabolic products developing in the working (exhausted) muscle. We were unable to establish the nature of these products. Biochemical investigations had shown that in the exhausted muscle a breakdown of nucleotides and formation of free purine bases place [3]. Taking into account the fact that nitrogen bases suppress the mitotic activity [2,5,9], we made attempts to abolish the inhibition of mitosis during muscular exertion by preliminary injection of sodium citrate. This preparation inhibits the breakdown of nucleotides and ATP in the muscles [4]. The laws of these experiments were not sufficiently consistent, and we were unable to come to definite conclusions.

The results of the experiments described above thus show that during short-lasting intensive muscular exertion the mitotic activity undergoes a marked decrease. These changes are caused by at least two factors: the initial decrease in the mitotic activity is apparently caused by the increased secretion of adrenaline, and the subsequent inhibition of cell division is caused by metabolic products liberated from the working (exhausted) muscle. Changes in the mitotic activity found during short-lasting muscular exertion enable us to understand certain features characterizing the pattern of mitoses in the animal body. For a complete analysis of the daily rhythm of cell division from all aspects, however, further studies concerning the changes in the mitotic activity during prolonged but moderate functional exertion are necessary. It is quite possible that under the latter conditions the cell divisions will be affected in another way than during short-lasting intensive exertion.

SUMMARY

Brief muscular exertions in mice lead to a marked reduction of mitotic activity in the corneal epithelium associated with the delay in the onset of mitosis. In adrenalectomized animals cellular mitosis is inhibited later than in control animals. The perfusion fluid passing through the vessels of the strained muscle inhibits the cleavage of frog eggs. The deceleration of the cleavage rate is connected mainly with the prolongation of interkinesis. The action of metabolites of the working muscle is nonspecific. At least two factors are involved in the reduction of mitotic activity during physical exertion: initial reduction of mitotic activity is caused by intensified adrenalin secretion, whereas subsequent inhibition of mitoses is induced by the metabolites of the working muscle.

LITERATURE CITED

1. I. A. Alov. Byull. Éksper. Biol. i Med. 48, 11 (1959) p. 107.
2. I. A. Alov. Vopr. Onkol. 5, 1 (1959) p. 7.
3. A. M. Gudina. Ukr. Biokhim. Zhurn. 28, 2 (1956) p. 217.
4. A. M. Gudina. Ukr. Biokhim. Zhurn. 29, 1 (1957) p. 71.

5. L. M. Ermolenko. Byull. Éksper. Biol. i Med., 12 (1957) p. 102.
6. C. M. Blumenfeld. Arch. Path. 38 (1944) p. 321.
7. W. S. Bullough. Proc. Roy. Soc. B. 135 (1948) pp. 212-233.
8. W. B. Cannon and S. W. Britton. Am. J. Physiol. 79 (1926-1927) p. 433.
9. M. Deysson, C. R. Acad. Sci. 239 (1954) p. 1835.
10. Idem, Bull. Soc. Bot. France 102 (1955) p. 9.
11. L. V. Heilbrunn and W. L. Wilson. Publ. Stat. Zool., 27 (Napoli, 1955) p. 295.

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