EXPERIMENTAL BIOLOGY

CELL DIVISION AND METABOLIC PROCESSES TAKING PLACE WITHIN THE ORGANISM

PART II. MITOTIC ACTIVITY AND BASAL METABOLISM

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In our previous paper [4] we described the results of a study of mitotic activity in relation to changes in blood carbohydrate content. Our findings gave definite evidence of the existence of a correlation between them. In view of this, and since carbohydrates are the substrates of oxidative processes in the tissues, we undertook the present experimental study with the object of ascertaining whether mitotic activity was dependent on the level of basal metabolism of the organism.

We reported in one of our earlier papers [5] that changing the environmental conditions of rats led to inhibition of mitotic activity, associated with considerable enhancement of gaseous metabolism. As these reactions faded out, i.e., as the animals became accustomed to their new surroundings, mitotic activity gradually rose to the normal levels, and oxygen uptake fell. These observations thus pointed to the existence of an inverse relationship between intensity of gaseous metabolism and mitotic activity. However, this antagonism, observed by us in relation to the reaction of the animals to environmental changes, may have been the result of the action of some third factor, functioning simultaneously, and participating in the achievement of the reaction. Such a factor might, for example, be adrenaline, the secretion of which by the adrenal glands is enhanced when the animals are in an excited state.

The question arose, in this connection, as to whether mitotic activity would be changed, and in what direction, in animals in which oxygen uptake was stabilized at a raised level, without involving change in environmental conditions. Does the intensity of cell division depend in any way on the basal metabolic rate?

EXPERIMENTAL METHOD

Our experiments were performed on male white mice which had been maintained for a number of days under standard conditions. We caused changes in their basal metabolism by applying the fully physiological procedure of segregating animals which had been members of a given group for a shorter or longer period, and placing them into separate cages. It is known from the work of a number of authors [1, 2, 3] that this causes a sustained rise in basal metabolism of certain mammals, including laboratory rodents. Since we had to study mitotic activity and oxygen uptake simultaneously, we conducted our experiments in gas-exchange chambers, specially constructed for the purpose (we used chambers of the type designed by N.I. Kalabukhov in our earlier experiments). The chambers were fitted with a device providing for the maintenance of an air-stream through them, and in this respect they did not differ from the usual respiratory exchange chambers; the only novel feature of our chambers was that they consisted of four separate chambers, in place of the usual single chamber, all four chambers working simultaneously. The mice were introduced into the respiratory chambers after they had been kept under standard conditions for some time. We measured respiratory exchange immediately after the

mice were placed in the chambers (they were then at once killed), and also after one and three days. In the latter two cases the air-tight roof of the chamber was replaced by wire netting when metabolic measurements were not being made, in order to ensure free access of air to the animals. The duration of the experiments involving measurement of oxygen intake was in all cases 30 minutes. The experiments were conducted during the fall and the winter, at room temperature (10-18°). Under such temperature conditions herd metabolic reactions are well developed [2, 3].

The oxygen intakes were calculated per kg live weight of the animal. Where the mice were kept in groups we calculated the oxygen intake per unit weight of the group taken as a whole. Mitotic activity was observed in fixed preparations of whole corneal epithelium. We took the mitotic index as being equal to the number of mitoses observed in 100 square fields of vision, corresponding to 1 mm^2 of corneal surface. We counted the number of mitoses, and recorded the numbers of each mitotic phase.

EXPERIMENTAL RESULTS

We performed 12 series of experiments on 96 animals. In six of the series we studied the correlation between mitotic activity and basal metabolism, while the other six served as controls of these six groups.

We killed eight mice of each of the first and second series, which resembled each other in every detail. Three mice, taken from the group of five, were distributed singly in separate chambers. The control group (five mice) remained together in a single chamber. All animals were killed immediately after completing measurement of respiratory exchange, i.e., 35-40 minutes after the members of the group had been segregated.

Respiratory exchange was measured in the third and fourth series after the mice had been kept in the respiratory chambers for some days.

In the fifth and sixth series the mice were kept in groups or singly for three days.

We performed control series of experiments, in order to assess the effect of the actual experiment of measuring gaseous metabolism on the mitotic activity of the groups of mice under investigation. In these series the animals were placed in the metabolic chambers as before, but respiratory exchange was not measured.

The mice of the seventh and eighth series were killed 35-40 minutes after having been placed in the respiratory chambers, after one day for the ninth and tenth series, and after three days for the eleventh and twelfth series.

The average results of mitotic counts for the corneal epithelium and of measurements of oxygen intake are presented in the table, covering all the series.

Our experiments gave very uniform results. Gaseous exchange was invariably greater in mice segregated singly for various lengths of time. Thus, if the mean oxygen intake per kg body weight was 756 ± 13 cm^3 (mean of all groups), the value found for mice kept in isolation for various lengths of time was 1403 ± 12 cm^3. The statistical significance of the differences between the respiratory exchange of the groups under comparison is such as to leave no doubt of the reality of the phenomenon.

As is evident from the numerical data presented in the table, the raised level of oxygen intake corresponds with a higher mitotic activity. In all 12 series the mitotic index of the corneal epithelium of isolated mice is much higher than for animals kept in groups. Statistical treatment of the data gives a sufficiently high degree of significance of the shifts in mitotic activity, both for each series separately and for all the series taken together.

Although the absolute values of the mean mitotic indices vary from experiment to experiment, the general shift in the number of mitoses observed in the corneas of solitary mice, as compared with the value found for those kept in groups, is found regularly. The column of figures representing mitotic activity of solitary mice differs considerably from that for mice kept in groups. The probability of this difference's being due to chance is less than 0.005.

The actual procedure of determining respiratory exchange had, in general, no appreciable effect on the significance of the observed differences between the experimental and the control series. The same shifts in mitotic activity were found in the blank experiments in which respiratory exchange was not measured as in those of the first six series.

Mitotic Activity of the Corneal Epithelium and Basal Metabolism of Mice Kept Singly or in Groups

Series No.	Duration of experiment	Animals kept together in a group		Animals segregated singly			
				mean mitotic index		mean oxygen intake (cm^3)	
		mean mitotic index	mean oxygen intake (cm^3)	in absolute figures	as % of control	in absolute figures	as % of control
1	40 min	130±13	736	161±7	124	1351±17	184
2	40 min	74±8	752	131±24	177	1396±56	186
3	24 hrs	113±14	805	247±60	219	1441±22	179
4	24 hrs	70±6	764	125±10	179	1396±6	183
5	72 hrs	122±7	782	159±5	130	1428±16	183
6	72 hrs	78±8	700	100±2	128	1404±42	201
7	40 min	40±5	–	115±43	287	–	–
8	40 min	52±11	–	113±10	217	–	–
9	24 hrs	109±5	–	145±5	134	–	–
10	24 hrs	146±10	–	187±11	128	–	–
11	72 hrs	83±13	–	176±20	212	–	–
12	72 hrs	103±10	–	161±5	156	–	–
	Mean	93±9	756±13	152±11	174±16	1403±12	186±3

We are thus fully justified in stating that all animals showing a sustained rise in basal metabolic rate are also distinguished by the high mitotic count of the corneal epithelium.

The figures representing relative changes in mitotic activity and in oxygen intake correspond fairly closely with each other: oxygen intake of solitary mice was raised by 86%, and mitotic index by 74%.

The observation that the mitotic count varies parallel with the basal metabolic rate after both long periods of isolation and after short ones, of the order of 35-40 minutes, suggests that not every relative increase in respiratory exchange taking place during a short period of time is necessarily associated with inhibition of mitosis of tissue cells, as might have been concluded from a study of the data of the previously cited paper [5].

The essential difference between our present experiments and our earlier ones is that we now transferred the animals of both the experimental and the control groups to the metabolic chambers. The reaction to their transfer to a novel environment should therefore have been the same in both groups of mice. It may hence be concluded that the inverse correlation between basal metabolism and mitotic activity is due to a reaction of the organism to novel environmental features. In those cases in which heightening of basal metabolism is not associated with this reaction it leads to an increase in the mitotic index.

We were not able to discern any significant or consistent differences between the relative proportions of the mitotic phases observed in the corneal epithelium of mice kept singly or in groups, the animals of the experimental and control groups being killed after having been kept in the metabolic chamber for the same lengths of time. This shows that the enhancement of mitotic activity of the corneal epithelium of animals having a high basal metabolic rate is the result of a more or less uniform and regular increase in the number of mitoses, affecting all the mitotic phases equally.

It would have been very desirable to compare the velocities of mitosis of the corneal epithelium of mice kept singly and in groups. Unfortunately, however, our attempts at assessing these velocities have not so far been successful, because of the experimental difficulties involved. Nevertheless, and irrespective of what may be the ultimate results of determination of velocities of mitosis at different basal metabolic rates, our results support the conclusion that the durations of mitosis and of the resting phase are in some way related to the basal metabolic rate. Intensification of metabolic processes, leading to a prolonged and sustained rise in the number of mitoses, determines the relative, and possibly also the absolute, shortening of the resting phase of the life cycle of the epithelial cells of the cornea.

This shortening of the resting phase appears to be due to intensification of synthetic processes in the cells.

SUMMARY

Data concerning the study of interrelationship between the mitotic activity of the corneal epithelium and the basal metabolic rate in mice are presented in this paper. Mice which were kept in individual cages and several mice kept together in one cage were used in this experiment. Oxygen consumption in mice kept in individual cages is permanently increased. The increased number of mitoses in the corneal epithelium corresponds to the rise in oxygen consumption by these animals. Increased number of mitoses is due to a more or less proportional increase of all stages of division. The authors came to the conclusion that the relative length of the interphase in the life cycle of a cell is decreased in animals with high basal metabolic rate.

LITERATURE CITED

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***See English translation.