CELLULAR MULTIPLICATION AND METABOLIC PROCESSES IN THE ORGANISM

COMMUNICATION I. MITOTIC ACTIVITY AND BLOOD SUGAR CONTENT

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Cellular multiplication, the most important factor in normal and pathological growth, is closely linked with the process of metabolism. However up to now the details of the interrelation of cellular division with metabolic processes in conditions of the entire organism have remained unclear.

One of the questions scarcely touched by investigation in this field is the question of the role of carbohydrate metabolism in the regulation of cellular division.

Fundamental importance in the process of mitotic division of the cell is attributed to glycolysis [3, 6]. There is no doubt that the period of preparation of the cell for mitosis, depending on the level of the synthetizing processes, is connected with the character and the intensity of carbohydrate metabolism. In experiments on mice, Bullough [1,3] found a connection between the sugar level in the blood and the mitotic activity of the epidermis of the ear. According to his findings, injection of starch, producing an increase in blood sugar content, considerably stimulates mitosis in the epidermis. In contrast to this upon fall in blood sugar content, produced by injections of insulin, suppression of mitoses is witnessed.

Bullough and also Bullough and Eisa [4] established a link between the daily dynamic of mitotic activity, on the one hand, and the daily fluctuations in blood sugar and also the tissue glycogen content, on the other.

However Laws [5] who studied the mitotic activity of the epidermis and carbohydrate metabolism in normal and tumorous mice was not able to confirm the findings of Bullough.

The conflicting published data and the need to clarify the role of carbohydrate metabolism in manifestations of cellular multiplication prompted us to undertake the present investigation. We set ourselves the task of studying the character of the interrelation of the blood sugar level and mitotic activity of the epithelium of the cornea.

The experiments were performed on white mice (chiefly on adult males). Before the experiments were conducted a group of mice chosen as far as possible for uniformity were kept a few days in completely identical conditions. Blood coagulation, in the determination of blood sugar, according to the method of Hagedorn and Jensen, was prevented by addition of potasium oxalate. At the same time the cornea was fixed in Carnoy fluid. Mitosis was calculated on the total corneal preparations. As the mitotic index we took the number of mitoses per 100 rectangular fields of vision corresponding in general complexity to 1 square millimeter of area of epithelium. In the present communication we present data obtained in 7 series of experiments on 95 animals.

First series: 6 days before the experiment 15 mice were placed in one cage. On the day of experiment at 10:10 a.m. the first mice were brought from the cage, decapitated and their cornea fixed for histological examination. At the same time blood specimens were taken for biochemical analysis. Immediately after sacrifice of the first 5 animals, lasting for 7 minutes, the following 5 mice were taken out and switched to another cage. In 15-25 minutes both groups of experimental animals, both those which had been switched and those

remaining in the former cage, were killed.

The second series of experiments was a repetition of the first, but with a slight modification, namely: the last two groups of mice (5 animals each) were killed at earlier stages – 6-15 minutes after the 5 control animals had been removed from the cage.

The third series was treated differently from the first two. The 15 mice kept in the cage on the day of the experiment were not taken out in fives but one at a time at three minute intervals.

The fourth series was analogous to the third: the group of 10 mice were taken out one at a time at two minute intervals.

In the fifth series of the experiments 20 mice were used. The animals were placed in two uniform cages with 10 mice in each. 10-15 minutes before sacrifice of the animals and fixation of the material the first (experimental) group was moved to another cage, the second group was subjected to no influence and served as control.

In the sixth and seventh series we investigated the influence of introduction of 0.5% solution of adenosintriphosphate (ATP), neutralized by caustic soda; an equal amount of neutralized distilled water was administered to the control animals. Whereupon in the sixth series the mice were killed 30-35 minutes after intraperitoneal injection of ATP and the water at a volume of 0.5 ml. In the seventh series the mice were killed at the same times but the ATP and water were introduced subcutaneously in a volume of 0.2 ml.

In all the series of experiments the influence applied, it would seem, was immaterial as, for example, change in the composition of the group of experimental animals and removal of them to another cage produced a perceptible fall in the number of mitoses in the cornea. These findings are in agreement with material previously published by us.

As is clear from the table presented by us decrease in the number of mitoses in all cases without exception was accompanied by an increase in blood sugar content. Statistical treatment of the numerical material, true, did not in all cases show the trustworthiness of the difference in the average values. However the identity of the shifts in mitotic activity and in blood sugar content, occurring in all the experimental groups under the influence of the varied effects, testify to the law-governed link between the investigated phenomena.

It is impossible to ignore the fact that this link was observed not only within the confines of a single experimental group, but upon comparison of the findings of the various experiments conducted at a different time and independently of each other.

Actually the largest number of mitoses in the corneas of the control groups (400) coincided with the minimum blood sugar content (113 mg%) and on the other hand the lowest mitotic index (239) was found in the group with the largest original blood sugar value (175 mg%).

Approximately the same correlation appeared upon comparison of the experimental groups of the various series one with the other. This gives grounds for considering that the factors which in the early stages of their action produced an increase in the blood sugar content simultaneously caused a decrease in the number of mitoses.

However, as was to be seen later, the link between the blood sugar content and mitotic activity was not identical.

Whereas in those series of experiments which we have just analyzed, if we compared not the mean indices, but the individual fluctuations of mitotic activity and blood sugar in each group then the picture of interrelation, as a rule, proves to be different and opposite to that which was noted by us above. In order to illustrate this point the data of some series of experiments is given in Fig. 1, 2 and 3.

As can be seen from these figures, in the majority of experimental groups, the curve of change in mitotic activity runs parallel with the fluctuations in blood sugar level. In other words, high activity was usually observed in those mice in which the sugar level was higher and vice-versa. Certain deviations from this orderly pattern were encountered, however in the majority of cases this pattern was sufficiently discernible.

It is necessary to stress that placing of the mice in a new environment, which occasioned shifts in the mean blood sugar content, did not have any appreciable effect, in the stages studied by us, on the character of the law-governed described relationship. On the other hand, in the first series in the original control group of

Mitotic Activity and Blood Sugar Level in Mice Subjected to Various Influences

Conditions of experiment	Series of experiment	Duration of influence in minutes	Mean mitotic activity		Mean blood sugar level in mg %	
			original (control) indices	findings obtained as a result of the influence	original findings	experimental findings
Change in number of animals in cage	first	15—20	336	307	142	168
Ditto	second	6—14	400	343	113	141
Transfer of mice to new cage	first	20—25	336	200	142	164
Ditto	second	10—15	400	363	113	151
Removal of mice from cage at 3 minute interval	third	42	339	289	136	144
Removal of mice from cage at 2 minute intervals	fourth	18	387	332	120	164
Transfer of mice from one cage to another	fifth	10—15	357	260	139	151
Intraperitoneal injection with ATP at dose of 0.5 ml of 0.5% solution	sixth	30—35	239	118	175	250
Subcutaneous introduction of ATP at dose of 0.2 ml of 0.5% solution	seventh	30	278	210	166	194

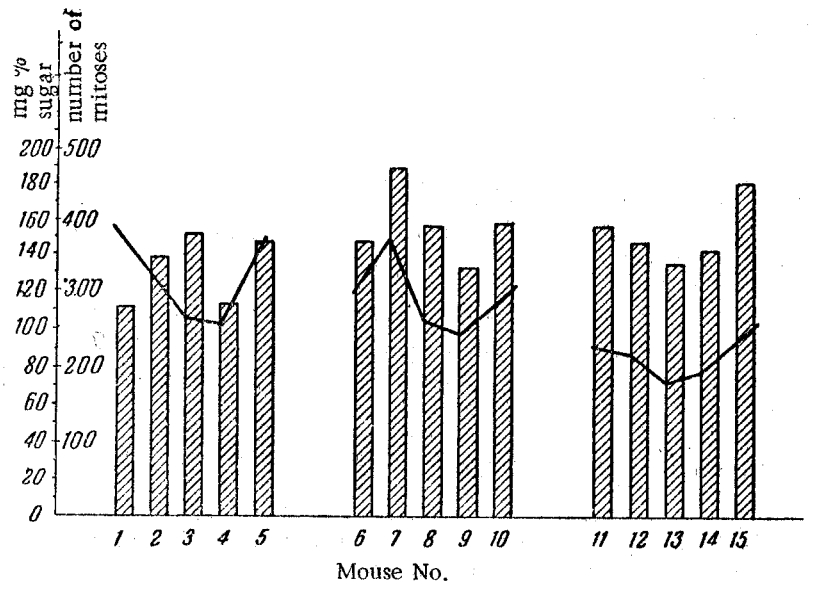

Fig. 1. Individual fluctuations in blood sugar content (columns) and mitotic activity (unbroken line) in experimental groups of mice of series I.

I) First group of mice.

II) Second group of mice, killed 20 minutes after the first one.

III) Third group of mice, killed, 25 minutes after the first.

animals no direct link between blood sugar and mitotic activity was observed. In the following two groups subjected to the above described influences this link was distinct, despite the fact that around this time a certain increase in the mean blood sugar content with a simultaneous reduction in mitotic activity occurred (especially in the third group of mice). A similar picture was observed in the third series in which the mice were killed one at a time at 3 minute intervals. As is clear from Fig. 2 the mutual relation between the blood sugar level and mitotic activity of the corneal epithelium, which was not apparent at first became absolutely distinct as from the 9th minute of the experiment. At the very end of the experiment lasting for 42 minutes this relationship was again somewhat disturbed. Probably, this was due to the fact that another parallel tendency existed and manifested itself: during the time of the experiment there was a wave-like, but steady decline in mitotic activity with a simultaneous increase in the blood sugar level.

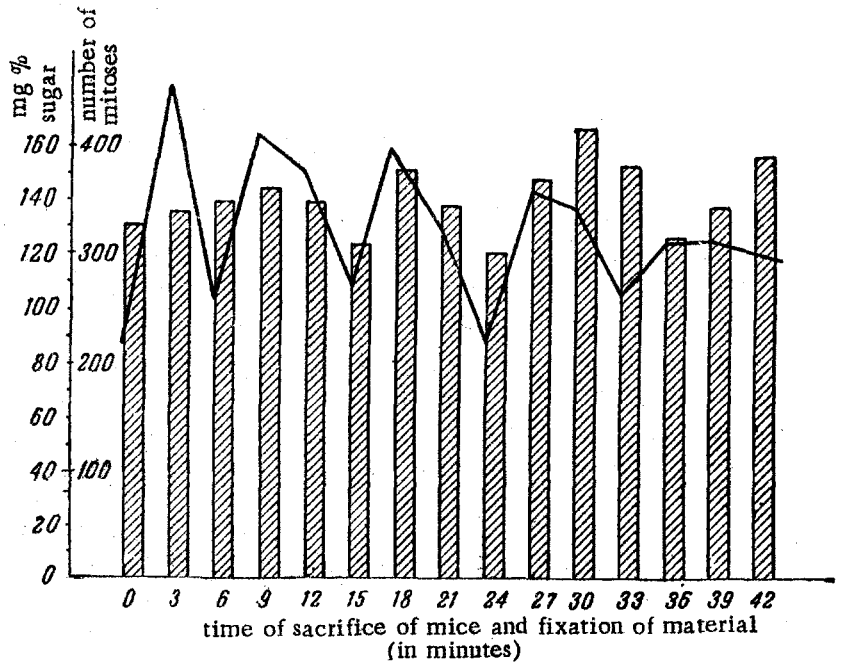

Fig. 2. Changes in blood sugar level (columns) and mitotic activity of corneal epithelium in mice (unbroken line) according to data of experimental series III.

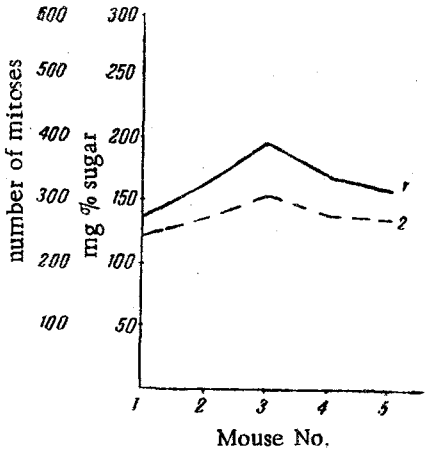

Fig. 3. Individual fluctuations in blood sugar content (1) and mitotic activity (2) in group of control mice of series VII.

Thus, the interrelation between the blood sugar and mitotic activity of the cells was not in all cases identical. There existed at least a dual kind of relationship between them. On the one hand the influences producing increased blood sugar determine a simultaneous decrease in the number of mitoses and, on the other, mitotic activity was higher in those animals in which the blood sugar concentrations were larger. These two tendencies may appear at the same time since the presence of one orderly relationship does not exclude the existence of another. It is fully possible that in other experimental conditions the dual mutual relationship between blood sugar and mitotic activity will reveal some other peculiarities.

In the experiments conducted by O. T. Movchan and Yu. P. Butnev on white rats the number of which was gradually reduced in 4 days (and not in a few minutes) a dual relationship between the blood sugar level and mitotic activity of the corneal epithelium was found. However this relationship was of a somewhat different character. The gradual decline in the number of rats in the cage was accompanied by a slow growth in blood sugar occuring simultaneously with an increase in the number of mitoses in the cornea. Together with this, comparison of the individual fluctuations in blood sugar and the number of mitoses did not disclose a direct link between them. The curve of individual fluctuations of mitotic activity proved to be a completely accurate (mirror-like) reflection of the curve of changes in the blood sugar level in separate animals (determination of blood sugar and mitotic activity in rats in this experiment was conducted after they had been kept for an hour in gas exchange chambers).

Thus, even in this experiment, in a somewhat different form, the same dual nature of the link between blood sugar and mitotic activity was identified. This lawful relationship is not peculiar to rats but is associated with the conditions of performance of the experiment analyzed and with protracted change in the composition of the group of rats and with their protracted stay in the gas exchange chambers.

The biological purpose of the existence of the dual link between mitotic multiplication of cells and blood carbohydrates at present remains unclear. It may well be that this duality is determined by the different role of blood sugar in the process of cellular division and in the periods of preparation for it. Both these stages in the life cycle of the cell (mitosis and interkinesis) differ from other each in their chemical, physiological and morphological features. One and the same factor acting in a different way in these stages in the life of the cell is bound to produce non-uniform changes in the mitotic activity of the tissue. However, this question requires special study. At present we consider it possible to affirm only the fact itself of a lawful link between mitotic activity and carbohydrate content. In this the link of mitotic activity with carbohydrate metabolism is characterized by great complexity. It is not identical even in one and the same experimental condition.

We consider that absence of an identical relationship between mitotic activity and blood sugar content has prevented certain investigators from seeing any general law-governed pattern in the mutual relations of these phenomena.

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