THE IMPORTANCE OF NUCLEIC ACID AND CARBOHYDRATE METABOLISM IN CELL DIVISION

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Reports in the literature on the importance of metabolic processes in cell division are very limited; in fact there are only a few histochemical investigations of variation in the nucleic acids of the dividing cell and some data on the importance of carbohydrate metabolism in this process. Several writers [1,2,3,6 and others] have shown that during division regular changes are observed in the content of ribonucleic (RNA) and desoxyribonucleic (DNA) acids in the cell. These investigations suggest that during mitosis there are definite changes in nucleic acid metabolism of the cell. However, from our point of view they do not provide adequate grounds for a conclusion on the importance of nuclein metabolism in cell division. Changes in nucleic acids at the time of mitosis may only accompany cell division and not be a fundamental biochemical process taking part in mitosis.

To investigate this problem we set up two series of experiments in which nucleic acid metabolism was disturbed and the mitotic activity of different organs was studied. The first series of experiment involved the use of 2,4 – dinitrophenol. This compound inhibits the formation of high-energy phosphate compounds of the nucleic acid, ATP, and phosphocreatine type, concerned in respiration [4,24]. Investigations on eggs of the sea-urchin showed that dinitrophenol blocked the entry of labelled phosphorus into the nucleic acid molecule.

EXPERIMENTAL METHODS

Our experiments were performed on the cornea of white mice. Mitotic activity was determined in the cornea of the left and right eye, in each case in an area of 3.3 mm^2. In both corneas the number of dividing cells and the ratio of the two first phases of mitosis to the next two phases was calculated (phase coefficient). Dinitrophenol drops were introduced into the right eye and physiological saline into the left eye. Comparing the number of mitoses in the cornea of the left and right eyes, we could judge changes in mitotic activity due to disturbance of nucleic acid phosphorylation. This experiment with dinitrophenol was preceded by a control group of observations in which the mitotic activity of the left and right eye of normal mice was determined. As these observations showed (10 experiments), the mean variation between the number of mitoses in the cornea of the left and right eye was 9%. Dinitrophenol was introduced in a concentration of 1 : 1000 1 hour and 1 hour 30 minutes before the animals were killed. The experimental and control animals were killed simultaneously at 17 hours.

EXPERIMENTAL RESULTS

In comparing the mitotic activity of the left (control) and right (experimental) corneas, attention is drawn first to the sharp increase in the phase coefficient. This is due to a considerable increase in the number of prophases and suggests inhibition of mitosis at this phase of division. On the other hand, a reduction in the total number of dividing cells was noted, by 31% on the average (Table 1). The experimental results are quite straightforward; they show that interference with intake of phosphorus into the nucleic acid molecule leads to reduction in the number of dividing cells and inhibition of mitosis at the prophase.

TABLE 1

Change in Mitotic Activity due to Dinitrophenol

Experiment No.	Time when animals killed	Number of mitoses and phase coefficient				Relation number of mitoses the same experiment to control (%)
		Right eye (experiment)		Left eye (control)		
200	After 1 hour 30 minutes	121	9.1	230	1.0	—48
201	The same	316	5.0	431	1.3	—26.7
202	» »	279	8.2	404	1.2	—31.1
203	» »	459	1.9	355	1.5	+24
204	» »	256	3.9	396	1.2	—36
207	After 1 hour	333	8.5	520	3.5	—35
208	The same	326	7.3	453	2.2	—28
209	» »	296	25.7	497	7.1	—40.4
210	» »	372	17.5	700	10.4	—46.8
211	» »	531	5.9	724	2.7	—26.7
	Mean	329	9.3	471	3.2	—31.5

In a second series of experiments we used adenine. This compound, like purine bases in general, sharply inhibits the activity of desoxyribonuclease [5]. Adenine sulphate was injected intraperitoneally into white mice 12 hours before they were killed. Control animals were injected with a corresponding volume of physiological saline. The mitotic activity was determined in preparations of the complete cornea in an area of 1.65 mm^2. The experimental results (Table 2) show that by injection of 4-6 mg of adenine a small rise in mitotic activity is observed in the retina. On injection of larger doses of the preparation (7-8 mg) a sharp reduction in mitotic activity takes a place in the corneal epithelium – by 18 times. Thus, blocking of desoxyribonuclease leads to delay in the onset of cell division (see Table 2).

In a third series of experiments we used acriflavine hydrochloride. This compound interferes with synthesis of nucleic acids, forming insoluble complexes with them and with nucleotides. Acriflavine hydrochloride was injected subcutaneously into the back of the mouse in a dose of 1.3 mg 3 hours before killing. The mitotic activity was determined in the corneal epithelium, the intestine and the tongue (Table 3).

TABLE 2

Change in Mitotic Activity in the Cornea due to Adenine *

Dose of adenine (mg)	Number of animals	Number of mitoses	Phase coefficient
(control)	13	365.0±36.0	1.35
4—6	20	433.2±45.8	1.47
7—8	12	20.2±6.1	6.86

* In contrast to the other experimental series the animals were killed at 10 a.m.

As can be seen from Table 3, disturbance of nucleic acid metabolism by acriflavine hydrochloride leads to considerable reduction in mitotic activity in all the situations studied. Some retardation of cell division at the prophase stage is noted, judging by the rise in the phase coefficient.

TABLE 3

The Effect of Acriflavine Hydrochloride on Cell Division

Group of animals	Number animals	Number mitoses and phase coefficient					
		Cornea		Intestine		Tongue	
Control	9	118.4±21	1,3	342.2±13.4	3,0	29.0±4.8	4.8
Experiment, after introduction of acriflavine hydrochloride	12	35.6±8	5,5	64.2±13.4	6,0	2.0±1.4	2.9

The second group of experiments in this series – using the local action of acriflavine hydrochloride – is still more significant. Acriflavine hydrochloride in a concentration of 1 : 100 was instilled on the cornea of the left eye of a white mouse (11 experiments) while drops of physiological saline were introduced into the right eye.

On comparing mitotic activity in the right and left corneas a reduction in the nunber of dividing cells on the average of 47% compared with the control group, can be seen. At the same time there is a marked increase in the phase coefficient (from 1.3 in the control group to 5.5 in the experimental). These changes in the coefficient were due to a considerable increase in the number of prophases and suggested retardation of mitosis at this stage of division.

All the experimental series show that the nucleic acid metabolism of the cell is one of the principal biochemical processes concerned in cell division. Interference with nucleic acid metabolism leads to almost complete disappearance of mitotic activity or to retardation of mitosis at the prophase stage. These findings enable us to support the view that the synthesis of thymonucleic acid in the dividing cell occurs not during the anaphase [15,25] but during the prophase [1,3,6,18,19,26].

Naturally, the processes of nucleic acid metabolism are not the only biochemical mechanisms concerned with cell division. Several observations have led to the suggestion that carbohydrate metabolism plays an important part in the mitotic process. These observations are mainly by Bullough and his co-workers [8,9,10,11, 12,13,14,15,16]. For several years these workers have asserted that changes in carbohydrate metabolism are the principal biochemical processes which determine the onset of cell division. In experiments with starch, insulin, glucose, azides, malonates, etc, these workers show considerable changes in mitotic activity. These findings, however, are disputed by Laws [21], whose experiments did not confirm the original findings of Bullough.

In order to study the importance of carbohydrate metabolism in the process of cell division we set up several experimental series with white mice and rats. We studied cell division in the cornea, tongue and ear. We judged mitotic activity by the number of dividing cells in a constant area (1.65 mm^2) and by the phase coefficient. Dividing cells were counted on 4 cuts, each 10 μ wide, taken every 100 μ from one area. Mitotic activity in the retina was determined, as in the previous group of experiments, in complete specimens. In the first experimental series mice were twice injected subcutaneously with 0.5 mg of 5% glucose solution 3 and 5 hours before being killed. In the second experimental series starch (20 mg) was injected subcutaneously 3 hours before the animals were killed. In the third series insulin, in doses of 0.01, 0.05 and 0.1 milli-units or triprotamine zinc insulin in a dose of 0.1 milli-unit was injected. In the insulin experiments the mitotic activity was determined 3 hours after injection, and in the triprotamine zinc insulin experiments, 24 hours after injection. In the fourth experimental series the pancreatic islet cells were blocked by alloxan. This series of experiments was performed on white rats injected subcutaneously with 200-250 mg of alloxan per 1 kg body weight. As

a criterion of blockade of the islet cells we used histological examination of the pancreas and blood sugar estimations (by Hagedorn-Jensen's method). We produced experimental diabetes in 9 out of the 25 animals. On the 5th day after injection of allozan, the mitotic activity of these animals was examined. The sugar content at this time increased from 100-120 mg % in the control animals to 350-370 mg % in the experimental animals. Each experimental group had its own control group of animals. The results of these experiments are given in Tables 4 and 5.

TABLE 4

Mitotic Acitivity after Injections of Glucose and Insulin

Group of animals	Number animals	Number of mitoses	Phase coefficient
Control	10	52.7±9.2	2.6
Experimental, after injection of glucose	13	71.3±8.7	2.8
Control	13	76.0±15.3	5.8
Experimental, after injection insulin	19	84.0±13.2	6.5
Control	4	28.0±5.0	5.8
Experimental, after injection triprotamine zinc insulin	6	36.8±2.8	6.0

As can be seen from Tables 4 and 5, carbohydrate administration, insulin injection and blockade of the islet cells have no effect on mitotic activity. The widest variations do not transgress normal limits. Statistical treatment of the results by the Fisher-Student procedure shows that the variation between the experimental and control groups of mice was not significant.

TABLE 5

Mitotic Activity after Injections of Starch and Alloxan

Group animals	Number animals	Number mitoses and phase coefficient					
		Cornea		Skin		Tongue	
Control	5	140.0±8.6	1.96	11.6±5.3	3.68	32.6±8.2	6.38
Experimental, after injection of starch	5	146.0±23.0	2.0	12.6±3.5	3.0	42.6±10.2	6.0
Control	13	151.0±17.6	1.8	17.7±2.9	3.6	85.0±9.9	4.4
Experimental, after injection of alloxan	9	116.1±13.0	1.9	22.0±4.7	11.2	81.1±14.4	5.0

In the fifth experimental series we tried to explain the relation between the level of the blood sugar and the number of mitoses. For this purpose we determined the mitotic activity in the epithelium of the cornea, tongue and skin and the blood sugar in 17 normal white mice and 21 rats.

As can be seen from Table 6, we could show no association between the blood sugar and the number of mitoses. Experiments with white rats gave the same result.

TABLE 6

Mitotic Activity and Blood Sugar in White Mice

Experiment No.	Blood sugar (mg%)	Number of mitoses		
		Cornea	Skin	Tongue
120	94	125	18	7
121	98	21	7	12
131	98	80	10	7
123	102	4	21	7
128	119	94	13	6
108	120	40	15	12
114	134	34	25	7
105	134	111	35	8
127	133	73	11	10
122	140	78	—	5
111	141	10	14	12
125	144	81	3	8
124	145	61	12	13
129	147	11	—	13
132	147	46	18	6
126	149	40	18	7
133	153	116	29	11
	r+mr	-0.04 ± 0.24	$+0.29 \pm 0.24$	$+0.20 \pm 0.23$

As the same time we investigated the effect on cell division of sodium fluoride which is known to suppress the process of glyoclytic phosphorylation. Drops of sodium fluoride were instilled into the right eye $1\frac{1}{2}$ hours before the mice were killed. A single instillation of this compound in concentrations of 1 : 1000 and 2.5 : 1000 had no effect on mitotic activity in the corneal epithelium (10 experiments).

Analagous experiments were performed with malonates (1 : 1000-1 : 10,000; $1\frac{1}{2}$ hours before killing), which are known to block succino-dehydrogenase thereby interfering with carbohydrate oxidation. However, in these experiments too, no change in mitotic activity occurred in the cornea.

Thus, experiments with loading carbohydrate, injecting insulin, blocking the islet cells, injecting fluorides and malonates and determining the blood sugar and the mitotic activity gave straightforward results. Changes in carbohydrate metabolism, both stimulatory and inhibitory, brought about no change in mitotic activity of tissue. If carbohydrate metabolism is important in preparing the cell for division, this role is evidently secondary or is effected through other links of intermediate metabolism. Possibly cells possess sufficient stocks of high-energy phosphate compounds to allow division to take place in spite of disordered carbohydrate metabolism.

The experiments described show that nucleic acid metabolism is one of the essential biochemical processes concerned in cell division. Interference with nucleic acid synthesis leads to delay in the onset of cell division or to inhibition of mitosis at the prophase. Changes in carbohydrate metabolism have no appreciable effect on cell division.

SUMMARY

Disturbance of the process of phosphorylation by 2, 4-dinitrophenol, exclusion of desoxyribonuclease by adenine, derangement of the synthesis of nucleic acids by tripaflavine cause pronounced decrease of mitotic activity in the corneal, intestinal and tongue epithelium. Nucleic metabolism of a cell is one of the fundamental metabolic processes which is connected with cell division. Changes of the carbohydrate metabolism

(experiments with carbohydrate load, administration of insulin, block of the islet apparatus by alloxan, introduction of fluorides and malonates) in the direction of stimulation as well as in the direction of decrease of this metabolism do not result in the change of mitotic activity of the tissues.

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