DAILY PERIODICITY OF MITOTIC DIVISION AND THE GLYCOGEN CONTENT OF THE WHITE RAT LIVER

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One of the factors influencing the daily mitotic rhythm is the functional activity of the organs [1-3, 5-10, 16]. Most authors have concluded that there is an inverse relationship between this quantity and the mitotic division of its cells. However results concerning the relationship between the mitotic activity of the liver and its glycogen-formation are contradictory. It has been shown [18] that division of the hepatic cells is associated with a reduction of their glycogen content; also [6] that at the period of maximum mitotic rate, in the rat liver there is a minimum amount of glycogen, and at the time of minimum mitosis, glycogen formation is maximal.

However it has been frequently asserted [11, 14, 15] that the maximal glycogen content occurs during the night and early morning, when the number of mitoses is maximal [4, 17].

The object of the present investigation has been to determine the nature of the relationship between liver glycogen formation and mitotic activity.

EXPERIMENTAL METHOD

The work was carried out on 232 white male rats weighing 160-180 g. For two weeks the animals were kept on natural food which was given at 9 a.m.

In the I set of experiments carried out on 128 rats the animals were first fasted for 24 h, and then at 10 a.m. were given food which was left in the cages for one h. From 10 a.m. onwards eight rats at a time were killed for 24 h. The first five killings were made at an interval of one h, and the others at two-h intervals.

In the II set of experiments made on 104 rats the animals were also fasted for one day, and then were allowed to take food for one h at 10 p.m.; the animals were killed at two-hourly intervals on the same day.

Pieces of the left liver lobe were fixed in Carnoy. In each case mitoses were counted in 500 fields of view, which included 13,500 nuclei of hepatic cells.

For histochemical determination of the glycogen, pieces of the same liver lobe were fixed by the method of Shabadash by use of fuchsin-sulfuric acid. Histological sections were measured on a microphotometer MF-4. The count was made on a linear galvanometer scale reading up to 100. The microphotometer magnified 21 times. We measured the absorption of the light over the whole section. Because of the gradual penetration of the fixative into the piece of tissue, the central portions of the section of the glycogen content appeared to be lower than at the periphery. Therefore photometric measurements were made separately at the periphery and at the center.

EXPERIMENTAL RESULTS

The results obtained in the I series of experiments (in the morning) are given in Table 1 and Fig. 1.

The curves of mitotic activity corresponds mainly to the diurnal curve showing the number of mitoses in the rat liver as obtained previously in this laboratory [4].

TABLE 1. Changes in the Mitotic Activity and in Light Absorption in Parenchymal Cells of the Liver When the Animals were Fed at 10 a.m.

Time at which the animals were killed	Mitotic coefficient (‰)	Light absorption	
		at periphery	at center
10	0,94	32	22
11	0,31	36	24
12	0,06		
13	0,28		
14	0,08	51	36
16	0,04	52	37
18	0,05	58	44
20	0,06		
22	0,04	55	39
24	0,19		
2	0,19	67	52
4	0,03	68	52
6	0,02	59	45
8	0,83	48	34
10	0,03	44	29
12	0,08		

A considerable number of mitoses (0.94‰) was found at 10 a.m. in a rat which had been fasted for 24 h. By 12 a.m. the number had fallen (0.06‰, P = 0.07), and remained depressed until 6 a.m. on the following day. By 8 a.m. the number had increased. A comparison of the mean number of mitoses occurring between 4 and 6 a.m. with those at 8 a.m. showed that the latter was significantly larger (P = 0.0001). By 10 a.m. on the next day the number had again fallen (P = 0.03).

Thus a 24-h period of fasting followed by feeding for one h at 10 a.m. did not influence the diurnal periodicity of mitosis. Similar results on the preservation of the diurnal mitotic rhythm with a similar arrangement of the experiment have been obtained in a study of pancreas [8], and of the epithelium of the mucous membrane of the fundus of the stomach in the same rat [9].

It must however be pointed out that at 8 a.m. on the second day the number of mitoses was less than the maximum number under normal feeding conditions [4].

The amount of glycogen in the hepatic cells reached a minimum value at 10 a.m., when the rats had been fasted for 24 h. After they had been fed the number gradually increased (between 10 and 14 h this increase was statistically significant, P = 0.0001), and reached a maximum in the night at 2 and 4 h. Starting at 6 h the amount of glycogen gradually fell (P = 0.0001).

Thus the minimum amount of glycogen was found in the fasted rats; it increased after they had taken food for one h, and was maintained at a high level for the next 18 h.

The results of the II series of experiments, in which the animals received food for one h (at 22 h) are shown in Table 2 and in Fig.2.

In this series of experiments, despite the fact that the animals were being fed in the evening, the curve of mitotic activity remained the same as in animals whose access to food was not restricted [4]. An increase in the number of mitoses between 22.00 hs and 6.00 hs, and a reduction in the number between 6.00 hs and 8.00 hs was nearly significant (P = 0.03 and 0.04). In the subsequent h, the numbers of mitoses varied irregularly.

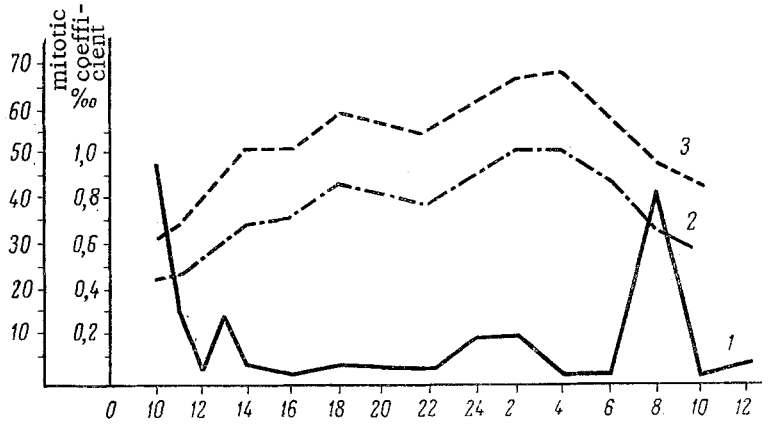

Fig. 1. Changes in mitotic activity and in light absorption in liver parenchymal cells when the animals were fed at 10 a.m. 1) Number of mitoses; 2) optical density in the central part of the section stained for glycogen; 3) optical density at the periphery of the section, stained for glycogen.

TABLE 2. Changes in the Mitotic Activity and Degree of Light Absorption in the Parenchymal Cells of the Liver when the Animals were Fed at 22 h

Time at which the animals were killed	Mitotic coefficient (%)	Light absorption	
		at periphery	at center
22	0,21	37	25
24	0,25	41	33
2	0,42	42	31
4	0,95	41	30
6	2,92	72	54
8	0,33	66	55
10	0,95	60	50
12	0,29	48	34
14	0,36	37	27
16	0,40	40	29
18	0,52	48	38
20	0,10	47	34
22	0,17	39	27

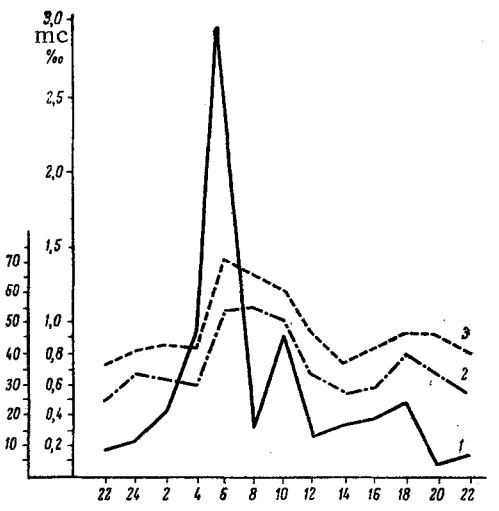

Fig. 2. Changes in the mitotic activity and degree of light absorption in the parenchymal cells of the liver when the animals were fed at 22 h. Indications as in Fig. 1.

Consequently a daily fast and a change in the h of feeding did not influence the diurnal rhythm of mitotic cell division.

The amount of glycogen in the fasting animals (at 22.00 hs), and in the fasted animals of the morning series was very small. It was only at eight h after feeding (at 6.00 hs) that the amount of glycogen rose sharply; after 10.00 hs the amount again fell and remained at a low level during the daytime. By 18 h there was a significant increase in the amount of glycogen ($P = 0.0001$), and it fell again 24 h after feeding (i.e., at 22.00 hr).

Consequently the low mitotic activity observed at 22.00 hs corresponds to a low glycogen content. At 6.00 hs, i.e., during the period of maximal mitotic activity the glycogen level also reached a maximum value. There was then a reduction in the number of mitoses and in the glycogen content of the hepatic cells.

From these results it can be seen that after an evening feed the diurnal changes in the mitotic activity and the glycogen content were of the same type.

Thus the nature of the daily periodicity of the mitoses is maintained whether the animals are fed in the morning or in the evening. At the same time the change in the glycogen content depends upon the time at which food is taken. The relationship between the position of glycogen in the liver and the time at which food is taken has been pointed out [12]. The greatest amount of glycogen was found six and twelve h after food had been taken; after 18 h the amount had fallen, and after 48 h had disappeared completely. It has also been pointed out [14, 15] that when the animals are fasted for a short time there is an alteration to the change of glycogen content. The authors of [13] have also pointed out the important part of nutrition in the diurnal cycle of glycogen formation; they obtained curves showing two peaks of change of glycogen content when rats, chickens, rabbits, or guinea pigs were fed at different times of day. A change in the times of feeding inevitably led to an alteration in the time at which glycogen was deposited in the liver. Apparently glycogen formation in the liver is more labile than the mitotic rate. A change in the time at which the animals are fed may easily alter glycogen deposition in the liver, whereas a longer time is required to change the diurnal mitotic rhythm.

The results we have obtained allow us to suggest an explanation of the contradiction between the diurnal changes of mitotic activity in the liver and the amount of glycogen deposited, as reported by other authors. It appears that the effect is to a large extent due to the fact that in their work the time at which the animals were fed was not related to the diurnal change of glycogen content.

When our preparations were examined it was found that when deposited at the period of maximum deposition liver glycogen is formed in large clearly stained clumps. At a period of the maximum deposition and when the amount is falling, the glycogen takes the form of fine weakly stained granules.

In preparations made in the evening experiment, when a maximum mitotic activity and glycogen content coincided we found in some cases that the glycogen in the dividing cells had been displaced towards the periphery, and in others that it was evenly distributed throughout the whole cell. Certain cells dividing mitotically contained less glycogen than those which were not dividing.

However it cannot be said that there is no glycogen in mitotically dividing cells or that the quantity is reduced by an amount related to the phase of cell division.

SUMMARY

Mitotic activity and glycogen content in the liver of white rats was measured one h after feeding at 10 a.m. or 10 p.m. after a 24-h fast. Irrespective of the time at which the rat was fed the maximal mitotic activity was found to occur between 6 and 8 a.m., and the minimal rate during the greater part of the 24 h. When the animals were fed in the morning the glycogen content rose gradually, reaching a maximum at a period when mitotic activity was minimal. With an evening feed the highest glycogen content occurred at 6 a.m., at the period of maximum mitotic rate. Subsequently both the number of mitoses and the glycogen content decreased.

Thus there was no direct relationship between glycogen formation and mitotic activity.

LITERATURE CITED

1. I. A. Alov, Byull. éksper. biol., No. 11, p. 107 (1949).
2. I. A. Alov, Tsitologiya, No. 3, p. 297 (1962).
3. I. A. Alov and N. V. Krasil'nikova, Dokl. AN SSSR, Vol. 142, p. 933 (1962).
4. V. N. Dobrokhotov, A. G. Babaeva, and A. G. Kurdyumova, Dokl. AN SSSR, Vol. 142, No. 2, p. 458 (1962).
5. M. K. Zahkarov, Byull. éksper, biol., No. 6, p. 81 (1961).
6. N. V. Krasil'nikova, Dokl. AN SSSR, Vol. 142, No. 5, p. 1165 (1962).
7. N. V. Krasil'nikova, Byull. éksper. biol., No. 11, p. 95 (1962).
8. I. V. Markelova and L. V. Sokolova, In book: Contributions to the 3rd Conference on Problems of Regeneration and Cell Division. [in Russian], Moscow, p. 106 (1962).
9. T. B. Timashkevich, In book: Contribution to the 3rd Conference on Problems of Regeneration and Cell Division. [in Russian], Moscow, p. 168 (1962).
10. C. M. Blumenfeld, Arch. Path., Vol. 38, p. 321 (1944).
11. H. W. Deane, Anat. Rec., Vol. 8,, p. 39 (1944).
12. H. W. Deane, F. B. Nesbett, and A. B. Hastings, Proc. Soc. exp. Biol. (N. Y.), Vol. 63, p. 401 (1946).
13. G. M. Higgins, J. I. Berkson, and E. Flock, Am. J. Physiol., Vol. 102, p. 673 (1932).
14. H. Holmgren and A. Swensson, Acta med. scand., Vol. 145, Suppl. 278, p. 71 (1953).
15. H. Holmgren and C. A. Ekman, Ibid., p. 46.
16. K. Peter, Z. Anat. Entwickl.-Gesch., Bed. 75, S. 506 (1925).
17. R. Peters, Z. Naturforsch., Bd. 17B, S. 164 (1962).
18. K. B. Roberts, H. W. Florey, and W. K. Joklik, Quart. J. exp. Physiol., Vol. 37, p. 239 (1952).

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