DIURNAL RHYTHM OF RNA AND GLYCOGEN IN FASTING ANIMALS

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The content of RNA and glycogen in the liver of fasting mice undergoes rhythmic fluctuations throughout the 24 h period similar to those observed under normal conditions. The RNA and glycogen levels fall significantly during fasting, although the diurnal rhythm is not thereby disturbed: the RNA concentration is lower by night than by day, while the glycogen content is higher at night, even after fasting for 62 h, than in the daytime after fasting for 24 h.

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Published data indicate a diurnal rhythm of RNA and DNA [1, 7, 12] and of glycogen [8, 10, 11]. We have found [3-6] a correlation in the intracellular changes in RNA and glycogen content in the liver during fever, after x-ray irradiation, in avitaminosis, and also under normal conditions throughout the 24 h.

In face of these facts, we have studied the intracellular RNA and glycogen metabolism in mice during the 24 h period but after preliminary fasting (from 24-62 h).

It has been reported [8, 9] that the glycogen content in the liver falls rapidly during fasting and disappears completely after 24 h. The endoplasmic reticulum undergoes important changes. In particular, there is a marked fall in the content, initially of RNA and, much later during fasting, of DNA also [2, 13, 14].

EXPERIMENTAL METHOD

Experiments were performed on 50 albino mice of the same sex kept under conditions of complete starvation for between 24 and 62 h. Healthy animals on a normal diet served as controls. Three series of experiments were performed. Liver tissue was fixed soon after decapitation in Carnoy's fluid. Paraffin sections (4 μ in thickness) from the liver of mice fasting for different periods and taken from animals of each series were placed on the same slide. In this way complete standardization of treatment of the sections (staining, washing, etc.), was ensured. The RNA and glycogen content was studied cytochemically (Brachet's method, staining with gallocyanin, Shabadash's method). Control sections were treated with ribonuclease and salivary ptyalin.

EXPERIMENTAL RESULTS

The following changes in the metabolic indices studied took place in the liver of the animals of series I fasting for between 24 and 44 h. After fasting for 24 h, i.e., at 1 p.m., the cytoplasm of the liver cells showed comparatively high basophilia, i.e., it contained a considerable quantity of RNA. Glycogen was absent in the liver at this time (Table 1). At later times and as fasting continued, the basophilia began to diminish to reach a very low level, especially at 3 a.m. During the daytime glycogen either failed to appear completely or was found in negligeable amounts as tiny or very tiny granules in individual cells. At 10 p.m. glycogen was found only in some cells lying close to the central vein in the liver lobule. However, its content at 3 a.m. was particularly interesting. At this time, when the animals had fasted for 40 h, the glycogen concentration rose appreciably. Glycogen granules were found in most cells and in large numbers.

In the experiments of series II the concentration of nucleic acids and glycogen was studied with effect from 36 h of starvation. The longest period of starvation in this series was 54 h. A study of preparations stained for RNA showed that the liver cells of these animals had a very low RNA content, although at these periods of fasting a tendency was observed for the basophilia to increase during the afternoon and evening.

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TABLE 1. Diurnal Rhythm of RNA and Glycogen Concentration in Liver of Fasting Mice

Cytochemical indices	Time of day			
	3 a.m. (after fasting for 42-62 h)	7-9 a.m. (after fasting for 52-48 h)	11 a.m.-3 p.m. (after fasting for 24-54 h)	9 a.m. (after fasting for 56 h) 10 p.m. (after fasting for 35 h)
RNA	After 40 h + After 42 h + After 62 h +	After 44 h ++ After 48 h + After 42 h +	After 24 h +++ After 54 h ++ After 48 h ++	After 35 h + After 36 h ++ After 56 h +
Glycogen	After 40 h +++ After 42 h +++ After 62 h +++	After 44 h + After 48 h + After 42 h +	After 24 h glycogen absent After 54 h glycogen absent After 48 and 50 h traces	After 35 h traces After 36 h glycogen absent After 56 h traces

Conventional signs. For RNA: +) denotes weak basophilia of cytoplasm, due to very few tiny granules; ++) denotes moderate basophilia with an appreciable increase in number of basophilic granules; +++) diffuse, well marked basophilia. For glycogen: +++) denotes that most cells have a moderate number of medium-sized and small granules. +) Indicates glycogen present only in cells near central vein and in small amount. Traces indicate glycogen granules scattered in negligeable numbers in individual cells.

In this series of experiments no glycogen was found in the cells after fasting for 36 h, the time being 9 p.m. However, at 3 a.m., i.e., after 42 h of fasting, glycogen was found in the liver of all animals of this series as tiny granules in many liver cells.

In the experiments of series III the animals fasted for between 42-62 h. The experiment began at 1 p.m. On the whole, the basophilia was much weaker in the liver cells of animals fasting for 42, 48, 56, and 62 h than in control animals on a normal diet. However, comparison of the RNA concentration in the liver cells at different times of day showed that in this series the basophilia was still higher between 1 and 3 p.m. than by night. The discovery of changes in the glycogen content was particularly intesting. Whereas at 1, 3, and 9 p.m., or after 48, 50, and 56 h of fasting respectively, either no glycogen could be detected by cytochemical methods or it was present merely as tiny granules in a negligible number of cells, more especially in the cells of the central part of the lobule, at 3 a.m., after fasting for 62 h, a larger reserve of glycogen was definitely found than was present during the afternoon and evening. At this time small granules were distributed comparatively regularly in many cells. Under fasting conditions the glycogen of the peripheral zone of the lobule is first used up, and not until later does glycogen disappear from the region close to the central vein.

Under fasting conditions, therefore, rhythmic changes take place in the RNA and glycogen concentration during the 24 h period. This diurnal rhythm shows the same tendency as in a normal physiological state: at night, despite prolonged fasting, the body finds its energy reserves in the form of carbohydrates deposited as glycogen in the liver. Consequently, the diurnal intracellular rhythm persists in fasting animals. The difference is that the amplitude of fluctuations in concentration of RNA and glycogen is progressively reduced.

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