DIURNAL RHYTHM OF PROTEIN METABOLISM IN HEPATOCYTES AND CUTANEOUS EPITHELIAL CELLS OF RATS WITH ACUTE RADIATION SICKNESS

V. I. Bulgak and L. I. Chekulaeva

UDC 616-001.28-036.11-092.9-07:
[616.36+616.5]-008.939.6-074"52"

Diurnal rhythms of protein metabolism in the hepatocytes and cells of the plantar epidermis of albino rats with acute radiation sickness were determined by autoradiography with methionine-S^{35}.

Previous investigations in the Laboratory of Experimental Histology of the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, by an autoradiographic method demonstrated a diurnal rhythm of protein metabolism in the cells of the adrenal cortex [7, 9], liver [2], and plantar epidermis [11] of rats. Comparison of the diurnal rhythms of protein metabolism and of mitotic activity showed that these two rhythms are synchronized in the cells of the liver and plantar epidermis, but not synchronized in the cells of the zona glomerulosa and zona fasciculata of the adrenal cortex.

The object of the investigation described below was to study diurnal changes in the level of protein metabolism after exposure to ionizing radiation.

EXPERIMENTAL METHOD

The experimental animals were 64 sexually mature male albino rats from the "Rappolovo" nursery, weighing 200-250 g, and the investigation was carried out at the beginning of February, when according to the records of the weather service the period of daylight in Leningrad lasts 9 h 45 min. The animals were kept under identical conditions and in natural illumination. The rats were fed and cleaned at the same time of day (from 8 to 9 A.M.). A single dose (600 R; 690 rad) of whole-body x-ray irradiation (RUM-11 apparatus) was given under standard conditions to 32 rats. Material from these animals was fixed on the 6th day after irradiation, i.e., at the height of development of acute radiation sickness. The control animals were 32 rats which were not irradiated. The irradiated and unirradiated rats were divided into 8 groups, with 4 animals in each group. The animals were sacrificed in groups at intervals of 3 h during the 24 h period. All animals received an injection of methionine-S^{35} in a dose of 1 μCi/g body weight 3 h before sacrifice. The material was fixed by Carnoy's method and embedded in paraffin wax. Autoradiographs were prepared with type R liquid emulsion (State Photographic Chemical Research Institute, USSR) by the method usually adopted in the laboratory [3]. Autoradiographs of the liver were exposed for 24 h and of the epidermis for 48 h. The number of tracks per unit area was thus less for the liver specimens than for the epidermis. The tracks were counted on the exposed autoradiographs: for the hepatocytes in 250 squares of the ocular micrometer grid, and for cells of the basal layer of the plantar epidermis in 100 squares. The area of 1 square corresponded to 50 μ^2 on the specimen. The numerical results were subjected to statistical analysis.

Laboratory of Experimental Histology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Department of Histology and Embryology, Kishinev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 10, pp. 42-44, October, 1972. Original article submitted September 21, 1971.

TABLE 1. Diurnal Fluctuations in Mean Number of S^{35} Tracts per Unit Area (50 μ^2) Above Hepatocytes (M ± m)

Time of day at which incorporation of S^{35} took place	Unirradiated rats	Irradiated rats
24—3	0,58±0,05	0,78±0,10
3—6	0,62±0,08	0,83±0,06
6—9	0,79±0,05	0,99±0,07
9—12	0,74±0,04	0,90±0,08
12—15	0,68±0,04	0,81±0,07
15—18	0,61±0,07	0,75±0,04
18—21	0,48±0,08	0,72±0,10
21—24	0,57±0,02	0,78±0,07
Mean values for 24-hour period	0,63±0,04	0,82±0,03

TABLE 2. Diurnal Fluctuations in Mean Number of S^{35} Tracts per Unit Area (50 μ^2) Above Basal Cells of Plantar Epithelium (M±m)

Time of day at which incorporation of S^{35} took place	Unirradiated rats	Irradiated rats
24—3	1,13±0,13	1,45±0,08
3—6	1,32±0,16	1,17±0,21
6—9	1,24±0,09	1,81±0,10
9—12	1,59±0,11	1,49±0,10
12—15	1,55±0,12	2,19±0,11
15—18	1,47±0,07	2,32±0,16
18—21	0,89±0,07	1,71±0,26
21—24	0,82±0,06	1,34±0,13
Mean values for 24-hour period	1,26±0,04	1,69±0,05

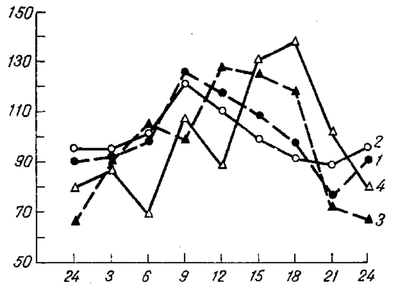

Fig. 1. Diurnal changes in intensity of protein metabolism in hepatocytes and basal cells of epidermis in unirradiated and irradiated rats. Ordinate, mean number of tracks per square (in percent of mean value for 24-h period); abscissa, time of day. 1) hepatocytes of unirradiated and 2) of irradiated rats; 3) plantar epidermis of unirradiated and 4) irradiated rats.

EXPERIMENTAL RESULTS

The experimental results are given in Tables 1 and 2. The statistics show that the accumulation of methionine in the hepatocytes of the control rats reached a maximum between 9 A.M. and 12 noon (Table 1). Starting from 3 P.M. the level of S^{35} incorporation gradually decreased, to reach a minimum by 9 P.M. Despite very slight deviations at subsequent times, this minimal number of tracks continued until 3 A.M. The difference in the number of tracks at 9 A.M. to 12 noon and at 9 P.M. to 3 A. M. is statistically significant ($P < 0.05$).

In the irradiated animals, as in the controls, the period of maximal incoporation of S^{35} occurred between 9 A.M. and noon. This was followed by slight fluctuations in the level of metabolism, but a definite decrease appeared only after 6 P.M. ($P < 0.05$). The mean level of incorporation of methionine into the liver cells of the irradiated rats for the 24-h period (0.82) was higher than for unirradiated rats (0.63). This difference is significant ($P < 0.01$).

In the control animals the minimal accumulation of S^{35} in the cells of the basal layer of the plantar epidermis occurred between 9 P.M. and midnight (Table 2). A significant increase in the incorporation began only at 6 A.M. ($P = 0.027$), reaching a maximum between noon and 6 P.M. In the irradiated rats minimal incorporation of methionine was observed between midnight and 6 A.M. The level then began to rise gradually, and by 9 A.M. the incorporation of S^{35} was significantly higher ($P = 0.024$), but the maximal number of tracts occurred between 3 and 6 P.M. The mean level of protein metabolism for the 24-h period in the unirradiated animals (1.26) was lower than in the irradiated rats (1.69). This difference is significant ($P < 0.05$).

Diurnal rhythms of protein metabolism in these two tissues also were clearly observed in the irradiated animals. Just as in the unirradiated animals, the curve of diurnal periodicity of S^{35} incorporation was unimodal in character (Fig. 1). Changes in the level of protein metabolism during the 24-h period in the irradiated rats were almost synchronous with the diurnal periodicity in the unirradiated animals, although the path of the curves was not completely identical in the two cases, as was previously pointed out by Truupyl'd [8]. Since radiation sickness does not follow a uniform course in all rats, the indices of protein metabolism in an organ such as the liver could be expected to vary more than in the epidermis of the skin. This was confirmed by the results obtained (Tables 1 and 2).

These observations are in agreement with the results of earlier studies which showed that diurnal rhythms of mitotic activity persist in acute radiation sickness [1, 4, 6, 8, 10]. They confirm the view [5] that mechanisms regulating the circadian rhythms of physiological processes in the body remain intact in acute radiation sickness.

LITERATURE CITED

1. V. I. Bulgak, Byull. Éksperim. Biol. i Med., No. 1, 90 (1965).
2. V. I. Bulgak, Tsitologiya, No. 6, 59 (1967).
3. L. N. Zhinkin, in: Radioactive Indicators in Histology [in Russian], Leningrad (1959), p. 5.
4. L. P. Kosichenko, Byull. Éksperim. Biol. i Med., No. 1, 114 (1963).
5. V. P. Mikhailov, in: Problems in Radiobiology and Clinical Roentgeno-Radiology [in Russian], Leningrad (1966), p. 72.
6. K. M. Svetikova, Tsitologiya, No. 2, 247 (1970).
7. A. Yu. Truupyl'd, Tsitologiya, No. 1, 103 (1966).
8. A. Yu. Truupyl'd, Regenerative Processes in the Adrenal Cortex in Acute Radiation Sickness. Author's Abstract of Candidate's Dissertation, Leningrad (1966).
9. A. Yu. Truupyl'd, Byull. Éksperim. Biol. i Med., No. 10, 83 (1967).
10. Yu. P. Khussar, Arkh. Anat., No. 10, 109 (1966).
11. L. I. Chekulaeva, Tsitologiya, No. 5, 632 (1969).