BIOPHYSICS AND BIOCHEMISTRY

Use of Assays for Chemiluminescence in the Examination of Animals Exposed to Ionizing Radiation

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Results are presented from chemiluminescence assays performed with samples of whole blood and wool from sheep exposed to gamma radiation in a dose of 200 or 600 R. Assays for barium sulfate-stimulated luminol-dependent chemiluminescence emitted by whole blood of the exposed sheep showed decreased amplitudes of chemiluminescence bursts shortly after irradiation, while assays for the lioluminescence of their wool sampled at different times within a week postirradiation and then stored for six months before the assay demonstrated higher burst amplitudes for samples from the animals irradiated with the higher dose and increases in burst amplitudes with increasing intervals between irradiation and sampling.

Key Words: *ionizing radiation; lioluminescence; chemiluminescence*

The rapid spread of sources of ionizing radiation has increased the risk of radiation disasters and accidental radiation exposures of living organisms, including farm animals [4], and has generated a need for reconstructing the radiation doses they may have received.

At present, consequences of radiation exposure are difficult to monitor. Simple, reproducible methods for assessing the impact of ionizing radiation on the environment are not yet available, making it urgent to develop ways in which its effects on living nature can be evaluated. One promising method appears to be recording of the chemiluminescence (CL) emitted by animal cells [2]. The purpose of the present study was to explore the possibility of using two kinds of CL assays to detect the dependence of radiation effects in animals on the radiation dose they have received and on the time elapsing after their exposure.

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MATERIALS AND METHODS

The study was conducted on 12 sheep of the Tsygai breed aged 2-4 years and weighing 32-46 kg. Nine of the sheep were exposed to uniform whole-body radiation by γ-quanta from a ^{137}Cs source in a dose of 200 or 600 R at a dose rate of 150 R/h. The other three sheep served as unexposed controls.

Barium sulfate-stimulated luminol-dependent CL (CL-L) emitted by whole blood taken from the jugular vein of the sheep was recorded before and at different times after their irradiation, using a procedure we described previously [3].

Wool was clipped from the back of the animals on days 1, 2, 3, 5, and 7 postirradiation. All wool samples, which were undyed (without traces of pigment), were stored under ordinary conditions until their assay 6 months after being clipped.

For the assay, 25 mg of wool were triturated with a porcelain pestle in a porcelain mortar for 10 min,

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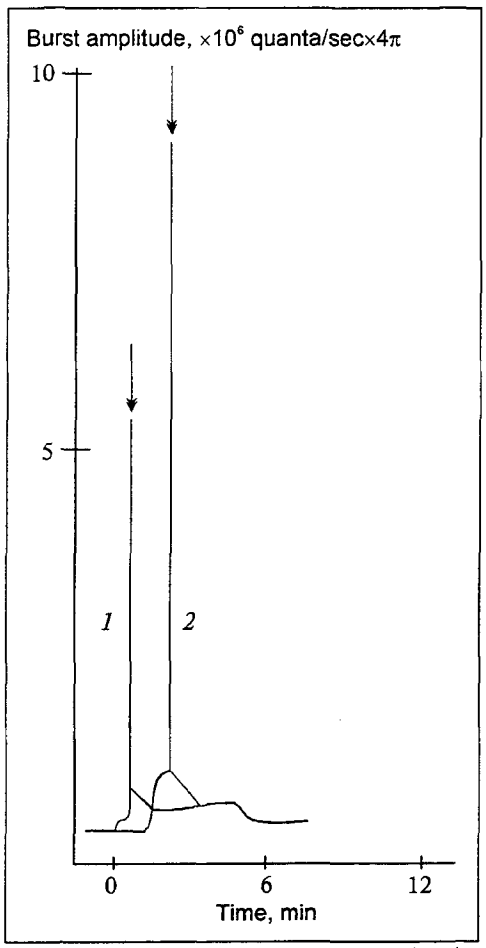

Fig. 1. Kinetics of LL bursts exhibited by sheep wool powder in response to the Na_2S solution added at the times indicated by the arrows. *1*) wool from a control sheep; 2) wool cut from a test sheep 7 days after radiation exposure.

and the resulting fine powder was spread on the bottom of a quartz cuvette which was placed in the dark chamber of a chemiluminometer. After a 2-min incubation of the cuvettes in the dark chamber, 1 ml of a saturated sodium sulfide (Na_2S) solution containing luminol was added to each cuvette via a tube without letting in any light [1]. This solution was prepared by adding 1.5 ml of 1.42×10^{-4} M luminol solution in dimethyl sulfoxide (2 mg/ml) to 10 ml of the saturated Na_2S solution. The addition of the luminol-containing Na_2S solution to the wool powder was followed by a fast burst of lioluminescence (LL), which was evaluated by measuring the burst amplitude (Fig. 1). All measurements were made at 37±0.5°C without special stirring of the samples.

RESULTS

As shown by clinical and laboratory methods, the sheep exposed to 200 or 600 R developed a radiation sickness that varied in severity and peaked on days 11-15 postirradiation. The dose of 600 R was lethal to all sheep.

The burst amplitude of the CL-L emitted by whole blood fell immediately after irradiation (Fig. 2). A decrease in CL intensity after radiation exposure is an indication of depressed phagocytic activity in animal blood [2].

Representative examples of the LL bursts exhibited by sheep wool after the luminol-containing Na_2S solution was added to wool powder samples are given in Fig. 1. It can be seen that no LL burst lasted for more than 30-40 sec and that the burst of wool from radiation-exposed sheep had a higher amplitude than that of wool from a control animal. The kinetics of the bursts was similar to the kinetics we had recorded for human hair samples after the addition of Na_2S solution without luminol [1]. The higher LL intensity after radiation exposure indicates that free radicals appeared in the wool under the impact of radiation and entered the solution from the solid phase to produce, upon recombination, an intensified burst of light [5-7].

The burst amplitude of LL was dose-dependent, being higher after the 600 R dose (Table 1). Wool samples from the sheep that died after this dose showed an increased LL intensity as early as on day 1 postirradiation and progressively higher intensities throughout the 7-day observation period, whereas those from the sheep exposed to 200 R did not exhibit enhanced LL until day 5. These findings confirm that ionizing radiation activates the generation of free radicals in wool.

TABLE 1. Burst Amplitudes of the LL (×10⁶ quanta/sec×4π) Emitted by Sheep Wool Clipped at Different Times after Radiation Exposure

Day after exposure	Radiation dose, R		
	0 (*n*=3)	200 (*n*=5)	600 (*n*=4)
1	7.7±0.3	7.0±0.4	8.6±1.2
2	7.5±0.2	7.1±0.5	9.2±0.5*,**
3	-	6.8±0.7	-
5	7.2±0.8	8.0±0.3	9.3±0.6*,**
7	7.6±0.6	8.8±0.5*	12.1±1.8*,**

Note. $p<0.05$: *relative to the control (unexposed) group; **relative to the group exposed to 200 R.

The increase in LL intensity with the time elapsing after irradiation can be ascribed to the development of degenerative changes in the integument. High radiation doses have been shown to cause damage to and impair the nutrition of hair bulbs [4]; in sheep this is manifested clinically in wool loss to the point where the animal may become completely bald.

The recording of LL emitted by wool can provide evidence of pathological changes occurring in the body long before the disease becomes clinically manifest. Furthermore, being a solid substrate, wool is able to retain for a long time information about the dose received by the animal. Indeed, the wool samples we used had been stored for 6 months at room temperature and yet proved suitable for assays.

In summary, an indication of the radiation dose received by an animal can be obtained by recording the CL-L of its whole blood shortly after the animal is exposed to ionizing radiation, while the recording of LL emitted by wool can identify exposed animals after a long interval.

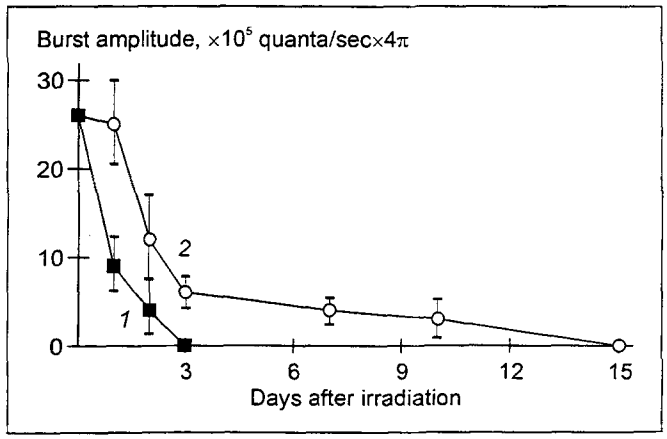

Fig. 2. Burst amplitude of the CL-L emitted by whole blood of sheep as a function of the time elapsing after their irradiation in a dose of 600 R (*1*) and 200 R (*2*).

REFERENCES

1. I. A. Alekhin, S. P. Babenko, S. N. Kraitor, *et al.*, *Atom. Energiya*, № 2, 91 (1982).
2. Yu. A. Vladimirov and M. P. Sherstnev, *Chemiluminescence of Animal Cells* [in Russian], Moscow (1989).
3. Yu. A. Vladimirov, M. P. Sherstnev, and A. P. Piryazev, *Biofizika*, № 6, 1051 (1989).
4. V. A. Kirshin, A. D. Belov, and V. A. Budarkov, *Veterinary Radiobiology* [in Russian], Moscow (1986).
5. V. V. Matyushkov, G. A. Mikhal'chenko, I. V. Yudin, *et al.*, in: *The Chemiluminescence Method in Biology and Medicine* [in Russian], Kiev (1978), p. 40.
6. V. V. Matyushkov, I. V. Yudin, and S. L. Panasyuk, *High-Energy Chemistry* [in Russian], № 2, 135 (1982).
7. W. Bogl and L. Heide, *Die Messung der Chemilumineszenz van Zimt-, Curry-, Paprika- und Milchpulver als Nachweis einer Behandlung mit ionisierenden Strahlen*, Neuherberg (1983).