

II. BIOENERGETYKA—BIOENERGETICS—БИОЭНЕРГЕТИКА

*Zofia Józwiak, Wanda Leyko*EFFECT OF GAMMA-IRRADIATION ON ENERGETIC METABOLISM
OF PORCINE BLOOD LYMPHOCYTES, PLATELETS AND ERYTHROCYTES *

Effect of gamma radiation (0.5-500 krad) on the levels of AMP, ADP and ATP in porcine blood lymphocytes, blood platelets and erythrocytes, 1^h after irradiation was studied. The applied gamma radiation in the dose range of 0.5-7 krad induced the same pattern of changes in the content of adenine nucleotides in all three cases studied. The experimental dose dependence of nucleotide levels in the range of 0.5-7 krad was in agreement with the applied mathematical calculations.

On the basis of the obtained data it was suggested that changes of nucleotides content in the above mentioned dose range are due to damage of the cell membranes.

Ionizing radiation creates numerous structural and metabolic disturbances in living cells, depending on their scope and range both on the radiation dose and on the kind of cell. As far as free nucleotides are concerned, the questions of the effect of ionizing radiation on cellular energy metabolism and of the role of energy-rich compounds in irradiated cells are still relatively poorly understood.

Studies on the effect of gamma radiation on erythrocytes in the dose-range of 10-50 krad revealed: a rapid potassium loss and sodium accumulation inside the red cell [12] hemolysis prevented by the addition of compounds not penetrating the erythrocyte

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membrane; 2-mercaptoethanol (MEG) and glutathione (GSH) to erythrocyte suspension prior to irradiation [7] and by SOD added extracellularly [2], a lack of radioprotective effect of intracellular SOD [3] and inhibition of $(\text{Na}^+, \text{K}^+, \text{Mg}^{2+})$ -ATPase starting from the dose of 5 krad, with only about 20% control activity at 20 krad [8].

MATERIAL AND METHODS

Lymphocytes, platelets and erythrocytes from porcine blood were used in these studies on the effect of radiation on nucleotides. Lymphocytes were obtained by the gelatine method of Malec et al [10] adjusted to own conditions. Blood was taken in 3% sodium citrate. Heparin was not used, taking into account the antiheparin activity of leukocytes, which could affect the level of free nucleotides in these cells. Leukocytes seem to take part in the process of binding heparin in vivo and its removal from the organism; this hypothesis is supported by an increase in the leukocyte count following heparin administration.

Filtered blood was added to 3% gelatin solution on 0.9% NaCl, at a temperature of 50-60°C and the mixture was left for 50-60 min at 37°C in order to sediment erythrocytes and granulocytes. The upper layer of the fluid, containing mainly lymphocytes was collected with a syringe. Lymphocytes were sedimented by centrifugation (1000 rpm, 10 min). Contaminating erythrocytes were removed by suspending the lymphocytes in a small volume of physiological saline and hemolysis by a modified method of Fallon et al. [6] consisting in the addition of chilled distilled water to the cell suspension, followed by the addition of chilled 3.6% NaCl solution to resorb the physiological saline concentration after 15 sec. The hemolysis was repeated, and the lymphocytes were suspended in veronal buffer, pH 7.2. Cell viability was tested by staining with 1% eosine solution (30 min, at 37°C). Under these conditions dead cells take up eosine while viable cells are not stained. Granulocyte contamination was estimated from smears stained according to Pappenheim. Erythrocyte contamination was determined by comparison of leukocyte and erythrocyte counts of the preparation from the same Bürker chamber.

The number of erythrocytes was divided by the number of lymphocytes.

Blood platelets were obtained by the method of Majda and Kotelba-Witkowska [9]. Blood was taken into 0.077 M EDTA, pH 7.4. In order to prevent adhesion and aggregation of platelets, all the preparation procedure was carried out in plastic vessels. The platelets were harvested by the method of successive centrifugations. First centrifugation (2,500 rpm, 8 min) yielded platelet-rich plasma. From further centrifugation of this plasma (2,500 rpm, 25 min) a compact sediment of platelets with an admixture of other morphotic elements and supernatant (platelet-poor plasma) was obtained. This supernatant was discarded, and the platelet sediment was suspended in physiological saline and centrifuged several times in order to separate the platelets from the contaminating erythrocytes and leukocytes. The purified platelet sediment was suspended in 0.9% NaCl and counted in a Thoma chamber following staining of the platelets with Rus-Ecker Blue. Cell viability was tested by staining with 1% Trypan Blue in 0.9% NaCl. Contamination of platelet preparations was estimated from smears stained according to Pappenheim.

Erythrocytes were obtained from ACD-treated blood and washed several times with 0.9% NaCl.

The acid-soluble fraction of control and irradiated samples was obtained by the method of Bartlett [1]. Separation of nucleotides was performed on Dowex 1X4, 200-400 mesh by the Mills method of continuous column chromatography in a formic acid-ammonium formate system. The obtained 3 ml fractions of the eluate were analysed spectrophotometrically at 260 nm. The contents of nucleotides were calculated basing on millimolar absorption coefficients, equal to 14.4 for adenine compounds, according to Mills.

Preparations of porcine blood lymphocytes platelets and erythrocytes were irradiated from a ^{60}Co gamma source at a dose-rate of 500 rad/min, in the dose-range of 0.5-500 krad. All estimations were carried out 1^{h} after irradiation.

Apart from the above mentioned calculations, the dose-response curves were approximated by orthogonal polynomials. This approach consists in the approximation of a curve passing through given points by the method of least squares, assuming function

given by sum of orthogonal polynomials Ralston [13]. In this method a polynomial is constructed:

$$Q_k = p(x) + r(x) \cdot q(x)$$

where $p(x)$ is a polynomial of order $L-1$, passing through given points for $k = 1, 2, \dots, L$, $q(x)$ is a polynomial of order $N-L$, minimizing the expression:

$$\sum_{k=L+1}^M w_k x_k^2 \frac{y_k - p(x_k)}{r(x_k)} - q(x_k)^2$$

where

$$r(x) = \prod_{k=1}^L (x - x_k)$$

Polynomials $p(x)$ and $q(x)$ are found by means of orthogonal polynomials, defined as follows:

$$\begin{aligned} \phi_0 &= 1 \\ \phi_n(x) - x \cdot \phi_{n-1}(x) &= \sum_{i=0}^{n-1} c_i \cdot \phi_i(x) \end{aligned}$$

The approximating polynomial is in turn, a linear combination of orthogonal polynomials $\phi_0, \phi_1 \dots \phi_N$

$$\phi_N(k) = \sum_{n=0}^N a_n P_n(x)$$

The sought polynomial is calculated finally using the formula

$$Q = \sum_{j=0}^n a_j x^j = a_0 + a_1 x + a_2 x^2 + \dots + a_n x^n$$

RESULTS

Checkup of lymphocyte viability demonstrated that dead cells constituted less than 2% of the total population studied. Lymphocytes constituted about 91% of all leukocytes of the preparation. Granulocyte contamination amounted to approximately 9%, erythrocyte contamination was equal to about 0.9 erythrocyte per

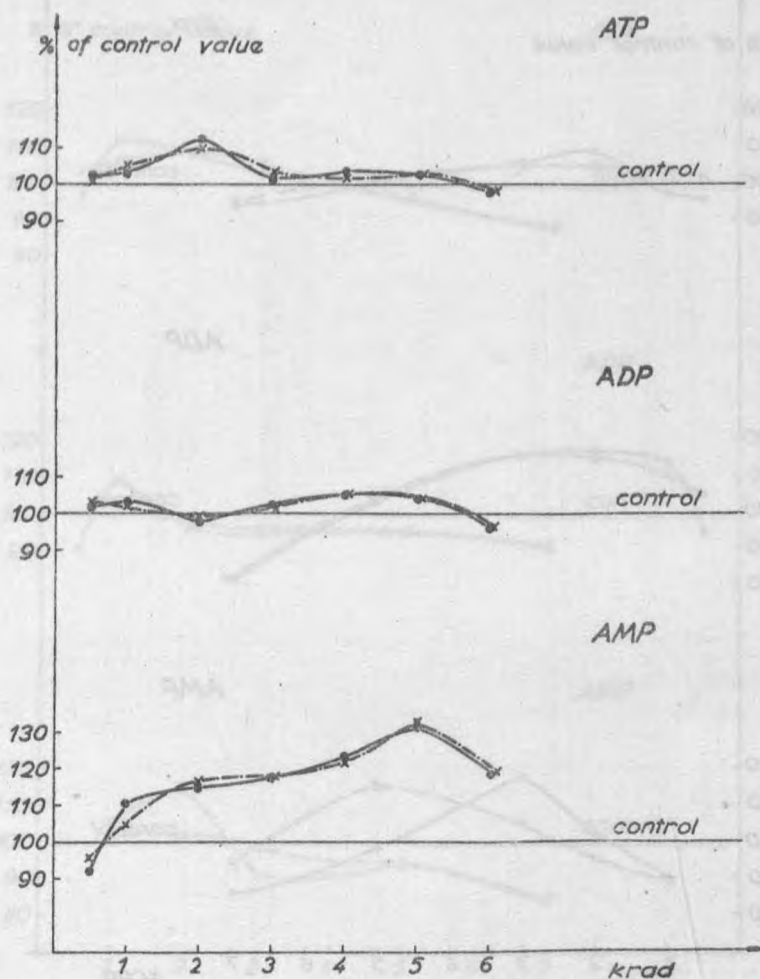


Fig. 1. Effect of gamma-irradiation (0.5-6 krad) on the levels of AMP, ADP and ATP in porcine blood erythrocytes, 1^h after irradiation, (—•—) experimental data, (x—•—x) mathematical data

Wpływ promieniowania gamma (0,5-6 krad) na zawartość AMP, ADP i ATP erytrocytów wieprzowych, 1 godz. po napromienianiu, (—•—) dane doświadczalne, (x—•—x) dane matematyczne

Влияние γ -излучения (0,5-6 Крад) на содержание АМФ, АДФ и АТФ в эритроцитах крови свиней, 1 час после облучения, (—•—) опытные данные, (x—•—x) вычисленные данные

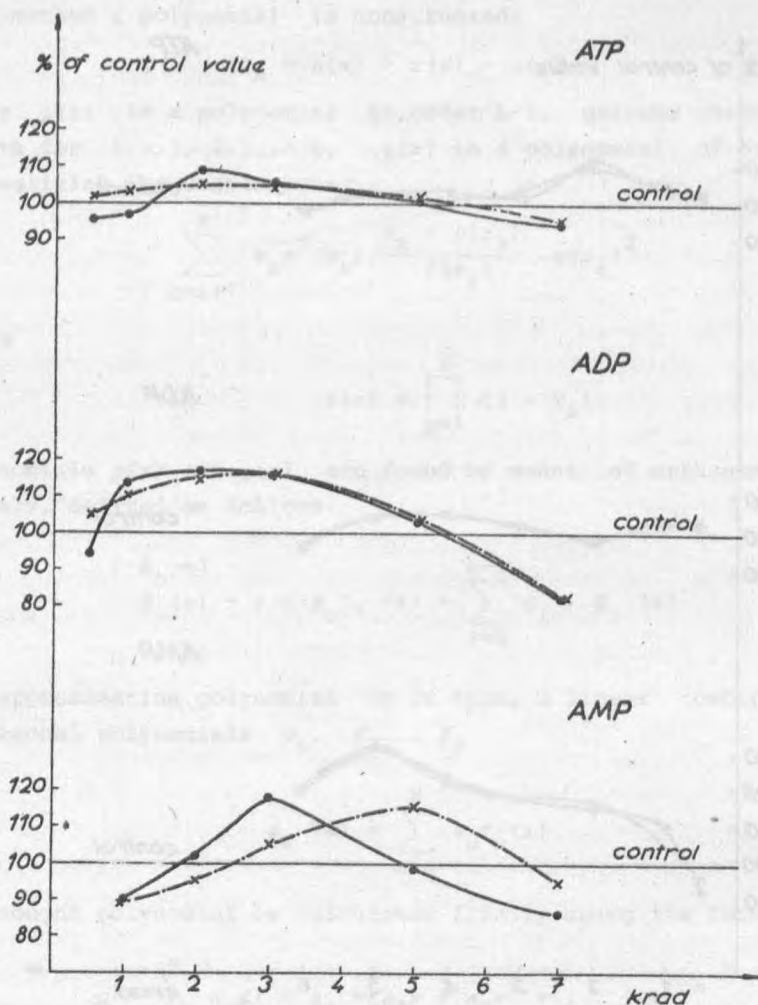


Fig. 2. Effect of gamma-irradiation (0.5-7 krad) on the levels of AMP, ADP and ATP in porcine blood lymphocytes, 1^h after irradiation, (—•—) experimental data, (x---x) mathematical data

Wpływ promieniowania gamma (0,5-7 krad) na zawartość AMP, ADP i ATP limfocytów wieprzowych, 1 godz. po napromienianiu, (—•—) dane doświadczalne, (x---x) dane matematyczne

Влияние γ -излучения (0,5-7 Крад) на содержание АМФ, АДФ и АТФ в лимфоцитах крови свиней, 1 час после облучения, (—•—) опытные данные, (x---x) вычисленные данные

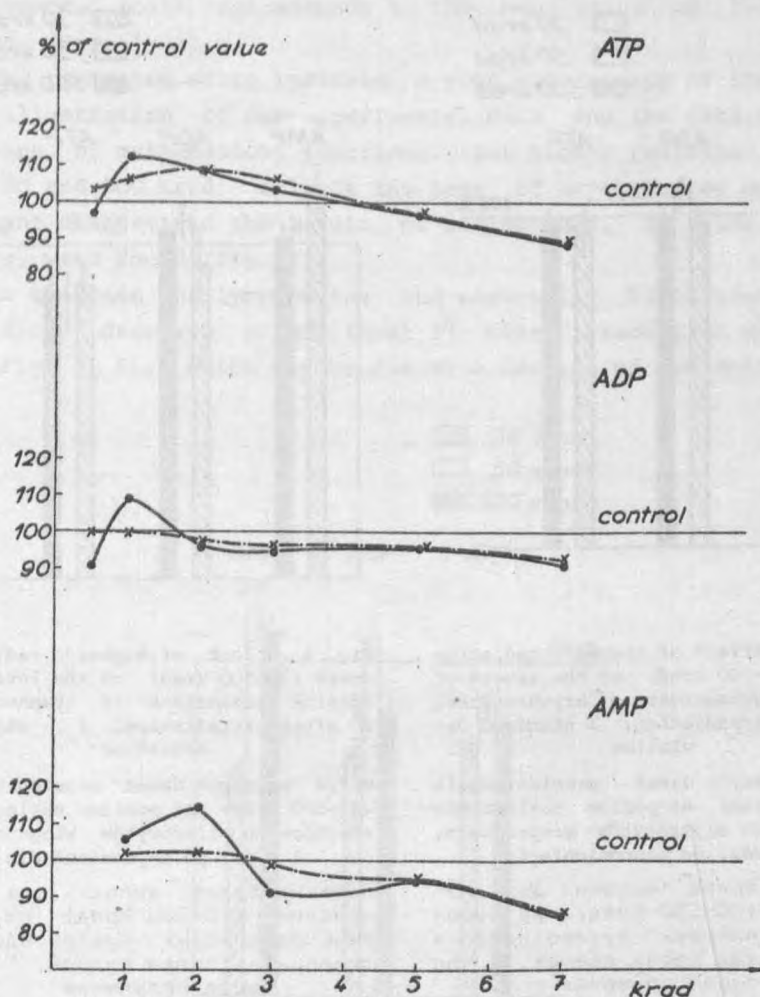


Fig. 3. Effect of gamma-irradiation (0.5-7 krad) on the levels of AMP, ADP and ATP in porcine blood platelets, 1^h after irradiation, (—•—) experimental data, (x—•—x) mathematical data

Wpływ promieniowania gamma (0,5-7 krad) na zawartość AMP, ADP i ATP płytek krwi wieprzowej, 1 godz. po napromienianiu, (—•—) dane doświadczalne, (x—•—x) dane matematyczne

Влияние γ -излучения (0,5-7 Крад) на содержание АМФ, АДФ и АТФ в кровяных пластинках свиней, 1 час после облучения, (—•—) опытные данные, (x—•—x) вычисленные данные

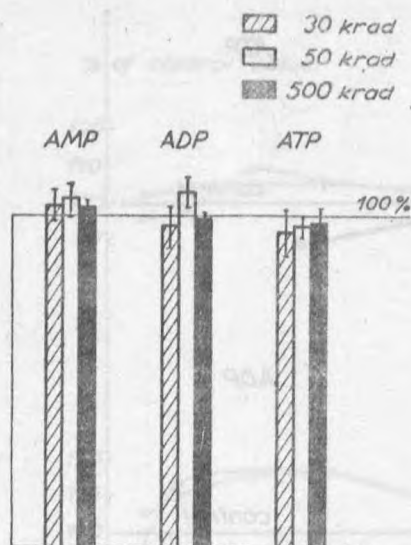


Fig. 4. Effect of higher radiation doses (30-500 krad) on the levels of adenine nucleotides of erythrocytes, 1^h after irradiation, 1 standard deviation

Wpływ wyższych dawek promieniowania (30-500 krad) na poziom nukleotydów adeninowych erytrocytów wieprzowych, 1 godz. po napromienianiu

Влияние более высоких доз излучения (30-500 Крад) на уровень адениновых нуклеотидов в эритроцитах крови свиней, 1 час после облучения

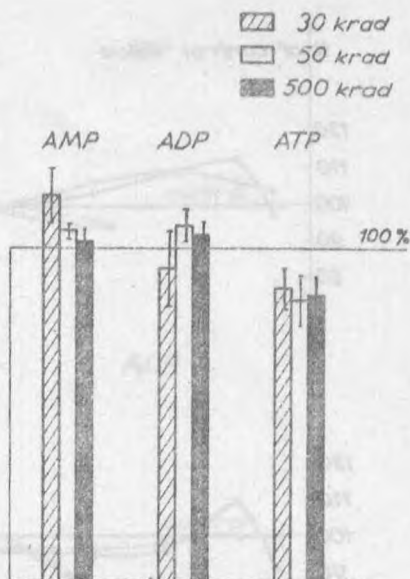


Fig. 5. Effect of higher radiation doses (30-500 krad) on the levels of adenine nucleotides of lymphocytes, 1^h after irradiation, 1 standard deviation

Wpływ wyższych dawek promieniowania (30-500 krad) na poziom nukleotydów adeninowych limfocytów wieprzowych, 1 godz. po napromienianiu

Влияние более высоких доз излучения (30-500 Крад) на уровень адениновых нуклеотидов в лимфоцитах крови свиней, 1 час после облучения

one lymphocyte. Since, the level of ATP in erythrocytes is about 20 times lower than the ATP level in lymphocytes, the portion of erythrocyte adenine nucleotides in the acid-soluble fraction of the preparations was less than 5%.

In the platelet preparations, erythrocyte contamination amounted to about 0.9% and the leukocyte contamination to about 0.6%.

Gamma radiation in the dose-range of 0.5-7 krad induced 1^h after irradiation the same pattern of changes in the content of

adenine nucleotides in all three types of cells studied. Each experimental point corresponds to the mean value of 5-8 estimations (Fig. 1-3).

The presented plots indicate a good consistency of the graphical illustration of our experimental data and the data computed by means of mathematical functions. For higher radiation doses (30, 50 and 500 krad) only in the case of erythrocytes no significant changes in the levels of nucleotides, 1^h after irradiation, were found (Fig. 4).

In the case of lymphocytes and especially blood platelets a significant decrease of ATP level 1^h after irradiation was observed (Fig. 5, 6), which may be due to a leakage of nucleotides.

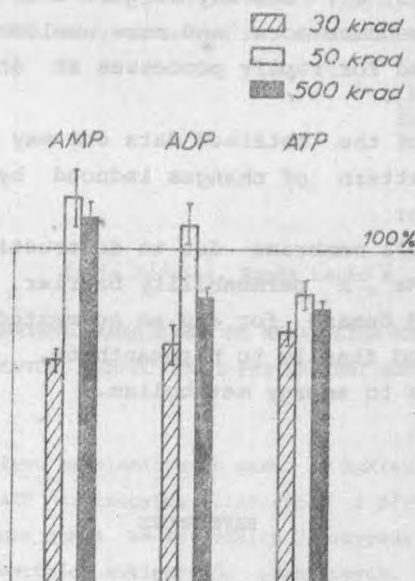


Fig. 6. Effect of higher radiation doses (30-500 krad) on the levels of adenine nucleotides of blood platelets, 1^h after irradiation, 1 standard deviation

Wpływ wyższych dawek promieniowania (30-500 krad) na poziom nukleotydów adeninowych płytek krwi wieprzowej, 1 godz. po napromienianiu

Влияние более высоких доз излучения (30-500 Крад) на уровень адениновых нуклеотидов в кровяных пластинках свиней, 1 час после облучения

On the basis of the obtained results one may conclude that the observed changes in the levels of adenine nucleotides follow

owing irradiation with doses up to 7 krad, can not involve repair processes nor disturbances of DNA metabolism since they are analogous in lymphocytes, blood platelets (lacking DNA) and erythrocytes (lacking DNA and cellular organelles). One may suggest therefore that the changes in the content of adenine nucleotides induced by the above mentioned doses are due to damage of the cell membranes.

As the adenine nucleotide feedback mechanism belong to mechanisms controlling the energy metabolism of erythrocytes, any changes in the content of these nucleotides evidence augmentation or diminution in the rate of metabolic processes and of active ion transport. Taking into account the inactivation of ATPase above 10 krad [4, 5, 8], one may suggest that the pool of adenine nucleotides becomes more and more useless for the cells and can not be utilized for repair processes at doses exceeding about 20 krad.

On the basis of the obtained data one may postulate the following sequence pattern of changes induced by doses of 0-10 krad of gamma radiation:

- damage to cell membrane due to destruction of SH groups and abolition of Na^+ , K^+ permeability barrier,
- an increased demand for and an augmented decomposition of ATP to ADP, AMP and finally to hypoxanthine,
- disturbances to energy metabolism.

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WPLYW PROMIENIOWANIA GAMMA NA METABOLIZM ENERGETYCZNY
ERYTROCYTÓW, LIMFOCYTÓW I PŁYTEK KRWI WIEPRZOWEJ

Praca dotyczy wpływu promieniowania gamma w zakresie dawek 0,5-500 krad na zawartość AMP, ADP i ATP erytrocytów, limfocytów i płytek krwi wieprzowej. Stosowane promieniowanie gamma we wszystkich 3 przypadkach wywołuje ten sam charakter zmian w zawartości nukleotydów adeninowych. Zależność zawartości nukleotydów od dawki promieniowania w zakresie 0,5-7 krad uzyskana eksperymentalnie jest wysoce zgodna z zastosowanymi obliczeniami matematycznymi. W oparciu o otrzymane wyniki sugeruje się, że popromienne zmiany zawartości nukleotydów adeninowych związane są z uszkodzeniami błon komórkowych.

Зофя Юзьвяк, Ванда Лейко

ВЛИЯНИЕ γ -ИЗЛУЧЕНИЯ НА ЭНЕРГЕТИЧЕСКИЙ МЕТАБОЛИЗМ
ЭРИТРОЦИТОВ, ЛИМФОЦИТОВ И КРОВЯНЫХ ПЛАСТИНОК
СВИНЕЙ

Статья касается влияния γ -излучения в диапазоне доз 0,5-500 Крад на содержание АМФ, АДФ и АТФ в эритроцитах, лимфоцитах и кровяных пластинках свиней. Во всех трех случаях применяемое излучение гамма вызывает такой же характер изменений содержания адениновых нуклеотидов. Обнаруженная в экспериментах зависимость содержания нуклеотидов от дозы излучения в диапазоне 0,5-7 Крад хорошо согласуется с применяемыми математическими расчетами. На основании полученных данных предполагается, что пострadiaционные изменения содержания адениновых нуклеотидов связаны с повреждением клеточных мембран.