



# Pharmacological Correction of the Results of Irradiation of the Parents for the Assimilation of Carbohydrates in the Small Intestine of Two Generations of their Offspring by the Help of Milk Thistle Fruits

**Objective:** The study of the assimilation of glucose and maltose in the small intestine of two generations of posterity of irradiated male rats received with a food the milled milk thistle fruits after irradiation.

**Methods:** Exposition of male rats performed once by the help of telegamma-setting "AGAT-R1", dose was 120 rad/min, field 20×20, distance from the source of irradiation to the field=75 cm, dose-0.5 Gy, exposure - 32 sec. Accumulating mucosa preparations (APS) produced by the method of Ugolev *et al.* APS incubated for 1 hour at  $t=37^{\circ}\text{C}$  in the oxygenated medium. As the incubation medium were used solutions 10 mmol/l of glucose and 5 mmol/l of maltose that were made on the Ringer solution pH 7.4. In all incubation medium the bile was added. The concentration of free glucose and M-glucose formed due to the hydrolysis of maltose was determined using method described in ref. Colorimetrically on photoelectrocolorimeter-CFC-2MP,  $\lambda=625$  nm. The statistical processing of the obtained data was conducted using "Primer Biostatistics" software.

**Results:** The 2<sup>nd</sup> generation of the offspring from the males, which received milk thistle in the food immediately after irradiation, showed the high activity of the transport system for the free glucose and system providing transport of M-glucose produced by the hydrolysis of maltose both. This activity is higher than in the corresponding groups of the 1<sup>st</sup> generation ( $p<0,0001$ ) and higher than corresponding data in all experimental groups of 2<sup>nd</sup> generation (except only group of the posterity of the male rats got milk thistle fruits before irradiation).

**Conclusion:** The use by predecessors of milk thistle after irradiation leads to the radio corrective effect on the systems of transport of glucose and maltose both exactly in second generation of their posterity.

**KEYWORDS:** Irradiation, posterity, small intestine, carbohydrates, pharmacological correction

## Introduction

The increased radiation background now is one of the condition for the existence of society. The use of nuclear energy, testing of nuclear weapons leads to pollution of the environment and, consequently, to the effects of radiation on the human body. High doses of radiation lead to acute radiation disease and rapid death of the organism. Small doses of radiation depletes the adaptive system of body and lead to long-term effects for the next generations such as slower growth, a violation of the digestive system, increased frequency of death after birth, heart disease, untransparency of lens, violation of memory and the capacity for associative thinking and increased fatigue [1]. Complete

elimination of the negative effects of exposure of predecessors in the population happens till 7-10<sup>th</sup> generation, but more than half of genetic diseases of the 1<sup>st</sup> generation appear in the 2<sup>nd</sup> [2]. Nevertheless, the process of research the phenomenon of transgenerational genomic instability in offspring of exposed parents is not completed yet [3-4]. Therefore, the search for ways of prevention and correction of disorders of the irradiated organism and its posterity continues.

In recent years, considerable attention is paid to herbal remedies, as a complex of the low-toxic agents with a broad spectrum of action and mild prolonged effect [5-7]. One of these herbal remedies is the fruits of the milk thistle,

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which thanks to the high content of membranotropic biologically active substances, is used to treat a variety of ailments - from arthritis and inflammation to cancer [8,9].

The last barrier between environment and intrinsic medium of organism is a small intestine – here we finish digestion of nutrients and absorb the final products into the blood. Therefore, any changing of the functional activity of small intestine because of environmental effects will lead to the disturbance of metabolic processes not only in tissues of the small intestine but also in the whole organism. The small intestine one of the first reacts to exposure: by the enterocytes desquamation, mucosal stratification, changes in the lipid membrane of enterocytes, violation of the conjugation of the processes of hydrolysis and transport of nutrients, leading to profound processes of assimilation disorders [10-12]. Therefore, the investigation of the effects of irradiation on the small intestine of the posterity of the exposure rats is still actually. Earlier *in vitro* conditions we have been shown the radio protective effect of total extract of milk thistle on the activity of the glucose transport system in the offspring of the 1<sup>st</sup> generation of irradiated hungry male rats [13]. It was interesting to find out whether this effect is stored *in vivo* – and later we have proved it for two generations of irradiated male rats, which got milled milk thistle fruits with a food before irradiation [14]. But no less, and perhaps more important than the prevention of effects of irradiation on the body is the correction of its effects - and from this point of view, it is interesting to study the radio corrective action of milk thistle fruits on the offspring of irradiated animals. Therefore, the aim of the work was the study of the assimilation of glucose and maltose in the small intestine of two generations of posterity of irradiated male rats received with a food the milled milk thistle fruits after irradiation.

### Materials and Methods

Experiments were conducted on two-month male rats of line of Vistar by mass 60-70 gram – posterity of the onetime irradiated male rats and intact female rats. Experimental groups were kept on the standard diet of vivarium and were deprived of meal during 18-24 hours before an experiment. Totally were used 7 groups of posterity: 1<sup>st</sup> intact; 2<sup>nd</sup> and 3<sup>rd</sup> descendants of two generations of irradiated hungry by a dose 0,5 Gy males and intact females, 4<sup>th</sup> and 5<sup>th</sup> descendants of two generations from males, that were irradiated by a dose 0,5 Gy after taking

meal (in satiety status) and intact females, 6<sup>th</sup> and 7<sup>th</sup> descendants of two generations from males, that were irradiated hungry by a dose 0,5 Gy and got 3 gr of milled milk thistle fruits with a food after irradiation, and intact females. Exposition of male rats performed once by the help of telegamma-setting “AGAT-R1”, dose was 120 rad/min, field 20×20, distance from the source of irradiation to the field=75 cm, dose-0.5 Gy, exposure - 32 sec. Accumulating mucosa preparations (APS) produced by the method of Ugolev *et al.* [15]. APS incubated for 1 hour at  $t=37^{\circ}\text{C}$  in the oxygenated medium. As the incubation medium were used solutions 10 mmol/l of glucose and 5 mmol/l of maltose that were made on the Ringer solution pH 7.4. In all incubation medium the bile was added. The concentration of free glucose and M-glucose formed due to the hydrolysis of maltose was determined using method described [16] colorimetrically on photoelectrocolorimeter – CFC-2MP,  $\lambda=625$  nm. The statistical processing of the obtained data was conducted using “Primer Biostatistics” software.

### Results and Discussion

Previously *in vitro* we had shown effects of irradiation of the exposed under different conditions male rats on their 1<sup>st</sup> generation of posterity [19]. It was shown the stabilization of activity of the processes of absorption of glucose in the small intestine of the 1<sup>st</sup> generation of the posterity of the male rats-predecessors which got milled milk thistle fruits with a food after the irradiation. Nevertheless, absolute data of these processes were significantly lower than data not only in the intact group but also in all experimental groups, yet parameters were within the active transport component (same). That is why especially interesting is the fact that the 2<sup>nd</sup> generation of the offspring from the males, which received milk thistle in the food immediately after irradiation, showed the high activity of the transport system for the free glucose and system providing transport of M-glucose produced by the hydrolysis of maltose both (TABLE 1). This activity is higher than in the corresponding groups of the 1<sup>st</sup> generation ( $p<0,0001$ ), higher than corresponding data in all experimental groups of 2<sup>nd</sup> generation (except only group of the posterity of the male rats got milk thistle fruits before irradiation). So, use of the milled milk thistle fruits with a food by male rats' immediately after irradiation leads to the stimulation of assimilation of carbohydrate substrates (both mono- and dimer) in the 2<sup>nd</sup>

**Table 1: Assimilation of substrates (mmol/l-mg) by the accumulation mucosa preparations from the mucosa of the small intestine of the 2-months-old rats – posterity of the 1-st and 2-nd generation of the irradiated by 0, 5 Gy hungry (I) and in satiety status (II) and in satiety status got milk thistle fruits before irradiation (III) and got milk thistle fruits after irradiation hungry (IV) males rats and intact females rats ( $M \pm m$ ).**

Group of Animals		Glucose	Maltose
I			
1. Intact	n =5	47,85 ± 5,59* 11,7%	42,71 ± 2,31* 5,4%
2. Posterity F1	n =5	<sup>x</sup> 42,25 ± 6,22* 14,7%	42,67 ± 1,95* 4,6%
3. Posterity F2	n =10	<sup>xx</sup> 40,35 ± 4,58* 11,4% $P_{1-3}=0,032$	31,77 ± 3,95* 12,4% $P_{1-3}=0,0001$ $P_{2-3}=0,0001$
II			
1. Intact	n =5	47,85 ± 5,59* 11,7%	42,71 ± 2,31* 5,4%
2. Posterity F1	n =5	<sup>x</sup> 51,97 ± 1,50** 3% $P_x = 0,013$	42,62 ± 1,94** 4,5%
3. Posterity F2	n =5	<sup>xx</sup> 33,47 ± 2,38** 7,1% $P_{1-3}=0,0001$ $P_{2-3}=0,0001$ $P_{xx}=0,018$	28,99 ± 2,66** 9,2% $P_{1-3}=0,0001$ $P_{2-3}=0,0001$
III			
1. Intact	n =5	47,85 ± 5,59* 11,7%	42,71 ± 2,31* 5,4%
2. Posterity F1	n =5	<sup>■</sup> 75,66 ± 3,89** 5%	<sup>■</sup> 70,59 ± 3,88** 5,5%
3. Posterity F2	n =5	<sup>■</sup> 120,49 ± 3,21*** 2,7% $P_{1-3}<0,0001$ $P_{2-3}<0,0001$	<sup>■</sup> 84,44 ± 1,70*** 2% $P_{1-3}=0,011$ $P_{2-3}<0,0001$
IV			
1. Intact	n=5	47,85 ± 5,59* 11,7%	42,71 ± 2,31* 5,4%
2. Posterity F1	n =5	<sup>■</sup> 24,97 ± 2,45* 9,8% $P_{■}=0,008$	<sup>■</sup> 19,26 ± 0,68* 3,5% $P_{■}=0,006$
3. Posterity F2	n =5	<sup>■</sup> 55,38 ± 4,90 8,8% $P_{1-2}=0,006$ $P_{2-3}<0,0001$ $P^{**}<0,0001$ $P^{\circ}=0,063$ $P^{\circ\circ}=0,004$	<sup>■</sup> 47,66 ± 5,22 11% $P_{1-2}<0,0001$ $P_{2-3}<0,0001$ $P^{**}<0,0001$ $P^{\circ}=0,034$ $P^{\circ\circ}=0,013$

Note. In all incubation mediums the rabbit bile was added.

Under the parameters of accumulation presents the percentage of the scatters from the middle value

\* - Data obtained earlier [17] used to compare

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x - the difference between the values in groups of the 1<sup>st</sup> generation of posterity from irradiated hungry (I) and in satiety status (II) male rats;

xx - the difference between the values in groups of the 2<sup>nd</sup> generation of posterity from irradiated hungry (I) and in satiety status (II) male rats

■ - the difference between the values in groups of the 1<sup>st</sup> generation of posterity from irradiated in satiety status got milk thistle fruits before irradiation (III) and got milk thistle fruits after irradiation hungry (IV) male rats and intact female rats

■ - the difference between the values in groups of the 2<sup>nd</sup> generation of posterity from irradiated in satiety status got milk thistle fruits before irradiation (III) and got milk thistle fruits after irradiation hungry (IV) male rats and intact female rats

° - the difference between the values in groups of the 2<sup>nd</sup> generation of posterity from irradiated hungry (I) and got milk thistle fruits after irradiation hungry (IV) male rats and intact female rats

°° - the difference between the values in groups of the 2<sup>nd</sup> generation of posterity from irradiated in satiety status (II) and got milk thistle fruits after irradiation hungry (IV) male rats and intact female rats

generation of their posterity – it means we detect the radio corrective effect of the remedy from milk thistle.

Due to analyzing of the results of all experimental groups we can detect that the absolute parameters of absorption of both carbohydrate substrates in  $F_2$  from the male rats got milk thistle after irradiation is significantly higher than the same group of the posterity  $F_2$  from the irradiated in satiety status male rats ( $p=0,004$  and  $p=0,013$  for free glucose and M-glucose correspondingly). Also is interesting the fact of the stability of obtained results: the scatters from the middle value in the  $F_2$  from the male rats got milk thistle after irradiation are less than in the same group of the posterity from the hungry irradiated male rats: for M-glucose - 11% versus 12,4% and for the free glucose – 8,8% versus 11,4%. In the last case the scatters from the middle value are less than even in the intact group on the background of the higher absolute parameters: 8,8% against 11,7% – it evidences the positive effect of the milk thistle on the organization of the transport system in the  $F_2$  in this group of irradiated rats and, as a result, about the elimination of the negative effects of irradiation.

Due to the analysis of absolute parameters in all experimental groups we found some interesting facts:

- In the posterities of the hungry irradiated male rats there were no difference between intact group and  $F_1$  for both substrates (free glucose and M-glucose), but it was authentic decreasing of the parameters in  $F_2$  for both substrates.
- In the posterities of the irradiated in satiety status male rats also there were no difference between intact group and  $F_1$  for both substrates, but in  $F_2$  for both substrates it was authentic decreasing of the parameters, and for the free glucose it was even less than in  $F_2$  from the hungry irradiated rats.
- In the posterities from the male rats, which got milk thistle before irradiation, the real parameters authentic increased

from the  $F_1$  to  $F_2$  and in  $F_2$  for both parameters were authentic higher than in the intact group. The results for  $F_2$  were the highest from all experimental groups for both substrates and there were fixed the lowest scatters from the middle value for all groups – it illustrates the stimulation and simultaneously stabilization by the milk thistle of the activity of the transport system for the free glucose and for the enzyme-transport conveyor for maltose as a precursor of M-glucose

- In the posterities from the male rats, which got milk thistle after irradiation, despite on the authentic decreasing of the rate of transport of both carbohydrate substrates in  $F_1$  in comparison with intact group (in 1,9 times for free glucose and in 2,2 times for M-glucose), in  $F_2$  it was authentic increasing of the parameters in comparison with  $F_1$ .

## Conclusions

Summing up we can say that the decreasing of the transport activity for both carbohydrates in  $F_2$  from the hungry and even from the in satiety status irradiated predecessors evidences the exhausting of adaptive systems responsible for the elimination of the effects of exposure of predecessors. In the same time the use of milk thistle before irradiation leads to the stable and even progressive radio protective effect in two generations of posterity, and the use by predecessors of milk thistle after irradiation leads to the radio corrective effect on the systems of transport of both substrates exactly in  $F_2$ .

Considering the fact of the long elimination of the effects of irradiation of predecessors in their posterity (the effects of irradiation of parents may be found in the 7-10<sup>th</sup> generation of their posterity) our results are encouraging from the point of view of the possibility to stop the chain of the transfer of radiation-induced injury of genome on the earlier stage and in this way make their elimination speedy. Taking into account these facts the role of milk thistle in this process is the defining and promising for further study.

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