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Secretory Activity Of The Pancreas And The State Of Enzyme Homeostasis In Hypokinesia And Radiation

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Annotation: *The problem of radiation emissions has acquired particular relevance throughout the world in recent decades. This is due to the widespread use of nuclear energy and radioactive substances, both for military purposes and in many sectors of the national economy in industry, agriculture, medicine, and research institutions. As a result, an increasing number of people are exposed to the harmful effects of ionizing radiation, which often lead to severe and irreversible consequences. [Fedorov V.P. and others., 2010)*

The pancreas synthesizes protein at a rate inaccessible to other organs except for the lactating breast, approximately 90% of the secretory protein is produced by acinous cells, and is an enzyme protein. In 1 hour, 20 mg of enzymes are synthesized per dry substance, or 107 enzyme molecules synthesize an acinocyte in 1 min. The pancreatic secret contains enzymes that hydrolyze almost all macronutrients consumed by humans - proteins, lipids and carbohydrates [Korotko G.F. 2017], i.e. they play an important role in digestion. The pancreas participates in enzyme homeostasis by increting and recreating them from the blood [Rotman S.S. 2002].

It is known that the digestive system is one of the most sensitive to the effects of radiation [Saada H.W., et.al ., 2009; Fischer C.J., Yosman P.C., 2008; Kim W.Y., et.al ., 2008], but the pancreas is considered a relatively radioresistant organ, since even at doses causing acute radiation sickness (700-1000 R), there are no significant morphological changes in it. A decrease in the secretion of pancreatic enzymes during in vivo irradiation may be the result of a weakening of stimulating effects at the level of their generation, as well as the conduction of signals in the chain of neurons of the meta-sympathetic ganglia of the gland [Yameguchi K. et.al ., 2000; Telbsz

A., Kovacs A.L., Somosy Z., 2002], as well as the result of inhibition of neurohumoral regulation processes expressed in a violation of the balance of adrenergic and cholinergic regulation in the gastrointestinal tract , the predominance of destructive processes ..

A decrease in physical activity and an increase in a sedentary lifestyle is a fundamental problem of modern medicine. Inactivity and hypokinesia, which leads to a stressful state, also adversely affects the functional activity of all organs and systems of the body, reducing overall resistance, lowering the natural mechanisms of its resistance. [Ivanauskene N.Yu., 2000; Izatulin A.V., et al., 2005].

Constant muscle activity is vital not only for the normal function of most systems and organs, i.e. the effector, but also the central nervous system. It is in the motor analyzer that all cortical afferences converge and converge, not only proprioreceptive, but also exteroreceptive and interoreceptive.

The changes observed from the digestive system in human and animal hypokinesia are combined by one concept, "hypokinetic syndrome of the digestive system." Changes in the activity of the organs of the gastrointestinal tract in hypokinesia are secondary. They depend mainly on the duration of the deficiency of activity leading to a decrease in energy consumption, bioenergetics and structural metabolism in the muscles, and a weakening of tonic impulses from the muscles. The use of radiation for therapeutic purposes in patients in bed rest creates the possibility of a combined effect of hypokinesia and radiation on humans. However, the patterns of the biological effect of radiation on the body in conditions of limited motor activity have not been sufficiently studied.

***Key words**: enzymes, amylase, lipase, common proteases, protein, blood, ferrum, Pancreatic homogenate.*

The purpose of the study. To study the secretory activity of the pancreas and enzyme homeostasis in hypokinesia and radiation.

Research objectives.The main objective of the research is to clarify the following relevant and unresolved issues:

- To study the enzyme-releasing activity of the pancreas and the activity of hydrolytic enzymes of the blood in intact rats.
- To establish the effect of the dose (1,2,4,6) of gamma radiation on the enzymatic activity of the pancreas:

-To study the effect of hypokinesia of various durations on the enzyme-releasing activity of the pancreas:

- To investigate the effect of hypokinesia of various durations on enzyme homeostasis:

-To establish the effect of hypokinesia of various durations of gamma radiation (4 Gray) on the enzyme-releasing activity of the pancreas;

- To investigate the effect of hypokinesia of different duration of gamma radiation (4 Gray) on enzyme homeostasis.

Materials and methods of research

The experiments were performed on 480 white laboratory mongrel male rats, weighing 180-200 g. Hypokinesia was modeled by placing rats in special small-sized pencil cases [Smirnov K.V., 1990; . for different time periods (1, 3, 10, 20, 30, 60 days,). The control for this group was the performance of rats in an ordinary cage, without restriction of movement. Total irradiation of rats with gamma quanta of 60Co was carried out on the "Ray" installation, the size of the irradiated field is 20x20cm, the skin focal length is 75 cm. The dose rate varied within 0.86-0.85 Gy/min. The absorbed doses were 1, 2, 4, 6 Grays. On 1, 3, 7, 10, 20, 30, 45 and 60 days after irradiation, the activity of enzymes in the homogenate of pancreatic tissue and in blood serum was studied. The

indicators of intact rats that were not exposed to any influences served as a control.

The rats were under ether anesthesia immediately before slaughter and they were slaughtered by decapitation, after which their blood was collected. After slaughter, the pancreas was extracted from the animals and homogenized by adding saline solution in a ratio of 1:10 to its mass. After that, it was filtered, the activity of hydrolytic enzymes was determined in the filtrate - amylase (by the Smidt-Roy method modified by A.M.Ugolev), lipase (by the Titz method), total proteolytic activity (by the Kunitz method) and total protein (by the Lowry method). The enzyme output was calculated by the ratio of activity to 1 g of pancreatic mass, examining the secretory activity of the pancreas and enzyme homeostasis in these rats.

In all other series of experiments after slaughter, identical studies of enzymes in pancreatic homogenate and in blood were carried out.

The resulting digital material was subjected to statistical processing on a Pentium –IV computer using a software package. The reliability of the differences (P) in the data was calculated using the t–Student criteria. The differences were considered significant at $P<0.05$.

The results showed that the amylolytic activity of 1460±56.0u/g was most pronounced in rat pancreatic homogenate.

In second place in terms of activity in rat pancreatic homogenate, total proteases are 230.0±6.1units/g. Proteolytic enzymes are synthesized and isolated by acinocytes in an inactive, zymogenic form in the form of trypsinogens, chymotrypsinogens, procarboxypeptidases, proelastases. Lipase activity in rat pancreatic homogenate is much less than that of previous enzymes. Its value is 70.1±3.1units/g..

Our results on blood enzymes in rats: amylase activity is quite high, it is equal to 560.0±11.0units/ml.In the blood, lipolytic activity is much lower (16.0±0.2u/ml) than its amylolytic activity.

Table-1
Activity of pancreatic homogenate enzymes in control rats in a calm state (M ±m)

№	Enzymes	Pancreatic Homogenate
1	Amylase	1460±56.0
2	Lipase	70.1±3.1
3	Common proteases	230.0±6.1
4	Total protein	4.5±0.3

In the blood, the pattern we noted is repeated in terms of the severity of the activity of amylase and lipase enzymes in the pancreatic homogenate.From this it can be seen that the pancreas plays a major role in the activity of blood enzymes, which is involved by incretion. The results showed that the correlation coefficients between enzyme activity in pancreatic tissue and blood are positive.The amylase activity index is r=0.64±0.13, and the lipase activity is r=0.48±0.16.

After gamma irradiation at doses of 1, 2, 4 Gray on day 3, amylolytic activity in pancreatic tissue decreased (Table 1). On days 7 and 10, the decrease in the activity of this enzyme reached its maximum values, i.e. this indicator became 20-40% less than the control indicators.

On day 60 after gamma irradiation at doses of 1 and 2 Gray, the amylolytic activity of pancreatic tissue reached

its initial values.

With an increase in the dose of gamma radiation, changes in amylase activity in the gland tissue were more pronounced. With gamma irradiation at a dose of 4 Gray, the amylolytic activity in the gland tissue decreased and remains at this level until 60 days after irradiation. When animals were irradiated with a dose of 6 Gy, amylase activity in pancreatic tissue decreased sharply after a day (by 28% below control).On the 3rd day after gamma irradiation, its activity slightly recovered (it became 13% lower than the control), but in the following days it became lower and lower and on the 30th day it became 70% lower than the control.

Amylolytic activity of pancreatic tissue under gamma irradiation (M±m, p<)

	1 Gray		**2 Gray**		**4 Gray**		**6 Gray**	
	control	**experience**	**control**	**experience**	**control**	**experience**	**control**	**experience**
1 day	1225±31	1141±41(0.1)	1010±3.5	1007±8(0.1)	1048±11.5	1071±3.7(0.1)	1090±1.5	790±2.5(0.001)
3 day	1241±45	1042±2.0(0.01)	1100±7.0	892±5(0.001)	910±6.8	848±1.5(0.001)	1160±4.1	1010±3.5(0.001)
7 day	1466±56	1193±36(0.01)	968±6.3	659±4(0.001)	917±6.3	676±1.4(0.001)	960±4.1	698±5.9(0.001)
10 day	1110±26	663±15(0.001)	1010±6.1	765.4±4(0.001)	928±6.4	820±8.9(0.001)	1010±4.8	548±2.4(0.001)
20 day	1210±37	887±2.0(0.001)	996±6.1	878±5(0.001)	990±6.6	950±1.6(0.001)	950±4.1	449±3.0(0.001)
30 day	1166±26	899±8.0(0.001)	928±6.8	736±4(0.001)	1001±6.1	820±2.15(0.001)	1009±4.6	308±3.6(0.001)
45 day	1172±34	966±16(0.001)	992±6.4	847±5(0.001)	928±6.3	823±1.0(0.001)	-	
60 day	1241±7	1225±0.6(0.1)	903±6.6	889±7(0.1)	960±6.4	800±1.8(0.001)	-	

Note: Amylolytic activity of x1000

Table 2.

Amylolytic activity of blood under gamma irradiation (M±m, p<)

	1 Грей		**2 Грей**		**4 Грей**		**6 Грей**	
	control	**experience**	**control**	**experience**	**control**	**experience**	**control**	**experience**
1 day	543.8±0.7	540±2.3(0.1)	536.7±1.5	500±5(0.001)	533±3.2	515±1.8(0.001)	511±1.4	261±1.1(0.001)
3 day	528.1±1.6	509±2.9(0.001)	564.1±1.6	532±5(0.001)	527±3.1	476±2.7(0.001)	514±1.5	238±1.3(0.001)
7 day	539.6±1.7	524±3.3(0.001)	548±2.1	460±9(0.001)	536±4.1	478,0±1.7(0.001)	540±1.6	184±1.2(0.001)
10 day	526.4±1.6	484±3.3(0.001)	529±6.1	462±2(0.001)	521±4.4	490±1.6(0.001)	526±1.7	160±0.7(0.001)

20 day	568.6±1.7	571±4.0(0.1)	567±1.6	510±3.6(0.001)	536±4.3	511.3±1.4(0.001)	528±1.6	111±1.4(0.001)
30 day	560.0±1.8	546±4.8(0.05)	564±1.8	546±1.4(0.001)	517±3.6	493.3±5.3(0.01)	540±1.7	86±1.2(0.001)
45 day	546.0±1.7	544±1.2(0.1)	580±1.9	581±5(0.1)	516±4.1	455.1±1.7(0.001)		
60 day	560.0±1.9	564±1.03(0.1)	570±1.7	575±1.8(0.1)	520±4.6	497±3.2(0.01)		

The direct action of inhibitory factors of acinocytes inhibits pancreatic secretion. In the pathways of the metasympathetic ganglia of the pancreatic neurons, the stimulating effect decreases and this leads to inhibition of gland secretion.This is an indirect factor.Often, the mechanisms of lowering pancreatic secretion are extraordinary (directly and indirectly) [Korotko G.F. 2005],

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A decrease in the activity of pancreatic enzymes may also be the result of a violation of enzyme protein synthesis.In irradiated cells, the synthesizing enzymes differ in the norms of kinetic parameters. Glucagon, somatostatin, enkephalin, calcitonin, releasing peptide, inhibitory gastric peptide, pancreatic polypeptide, corticotropin peptide and noadrenaline are inhibitors of the secretion of acinar enzymes.
A dose-dependent decrease in blood amylase activity was observed in experimental rats after gamma irradiation (Table 2).

With an increase in the radiation dose, there is a more pronounced decrease in the amylolytic activity of the blood, at a dose of 1 Gray by 2.5-8%, 2 Gray by 3-16%, 4 Gray by 5-12%, 6 Gray by 50-84% below control. Dose-dependent changes in lipase activity in pancreatic tissue and blood were obtained under gamma irradiation.

A decrease in the amylolytic activity of the blood is associated with some factors.Firstly, radiation inhibits pancreatic secretion, destroys hemodynamics, permeability of histohematic barriers, and the structure of capillary epithelium [Fedorov V.P., et al., 2000; Fedorov V.P. va.,2010; Gunadeva O.P. va., 2009, Yerofeeva L.M., 2008] and determines the number of blood enzymes.

The most radiation-sensitive endotheliocytes in the antinatal period of the body, when exposed to gamma radiation at doses of 0,5,1,0 and 2G, lead to insufficiency of functional organs and destruction of its structure.
At doses of 1 and 2 Gray, lipolytic activity in the homogenate of gland tissue and blood remained at the level of the initial values (Tables 3, 4).This means that these doses do not affect the secretion of lipase by the pancreas

and its secretion into the blood.
With an increase in the dose to 4 Gray, the activity of lipase in the gland tissue on the next day of gamma irradiation decreased approximately twice, on the tenth day after irradiation, its activity became 3 times lower than the initial values.At 60 days of follow-up, lipolytic activity in pancreatic tissue also remained much lower than the control parameters.

At a dose of 6 Gray, the lipolytic activity of the tissue on the next day after irradiation decreased by about 3 times, on 20-30 days this indicator became 4 times lower than the initial values.

Similar changes were observed in the lipolytic activity of blood under gamma irradiation at doses of 4 and 6 Gray.

A day after gamma irradiation at a dose of 4 Gray, the lipolytic activity of the blood decreased by 30%.. On day 45 after irradiation, a more pronounced decrease was observed, that is, lipolytic activity became 25-27% lower from the initial values, on day 60 it did not recover to the initial values, remained 15% below its level When experimental animals were given gamma radiation at a dose of 6 Gray, a wave-like change in blood lipolytic activity was observed. The day after irradiation, lipase activity in the blood decreased by 6%, after 3 days it became 25%, on day 7 48%- and on day 30 12% below control. The results prove that as radiation doses increase, rapid, then slow synthesizing enzyme enzymopathy occurs. .

The change in the overall proteolytic activity of pancreatic tissue also depended on the dose of gamma radiation. With gamma irradiation at a dose of 1 Gray, on the tenth day of the experiment, the total proteolytic activity of the gland tissue decreased by 18%, and on the twentieth day returned to its initial values. At a dose of 2 Gray, a different pattern of changes in protease activity in pancreatic tissue was observed. At the beginning, it decreased by 37% and then gradually, on the 45th day of the experiment, it returned to the initial values.With gamma irradiation at 4 Gray on the next day of the experiment, the proteolytic activity in the gland tissue decreased by 13%, from the 20th to the 60th day of the experiment, its activity became about 4 times lower than the initial level.When the animals were irradiated at a dose of 6 Gy, the next day the activity of proteases in the gland tissue decreased by 30% and in the following days of the experiment its activity decreased more and more, on the 30th day of the experiment it became 2 times lower than the control.

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Table 3.
The effect of hypokinesia and radiation (4 Gray) on amylolytic activity (M ± m; p<)

Duration of hypokinesia	**Blood**		**Pancreatic homogenate.**	
	Control	**exprince**	**Control**	**exprince**
1 **day**	$\frac{439 \pm 3.4}{100}$	$\frac{501 \pm 1.0(0.001)}{114 \pm 0.6(0.001)}$	$\frac{1210 \pm 17}{100}$	$\frac{1452 \pm 20(0.001)}{120 \pm 2(0.001)}$
3 **day**	$\frac{420 \pm 3.1}{100}$	$\frac{490 \pm 1.4(0.001)}{117 \pm 0.7(0.001)}$	$\frac{1186 \pm 16}{100}$	$\frac{1813 \pm 24(0.001)}{152 \pm 3(0.001)}$
10 **day**	$\frac{436 \pm 3.6}{100}$	$\frac{632 \pm 1.5(0.001)}{145 \pm 1.6(0.001)}$	$\frac{1127 \pm 16}{100}$	$\frac{3230 \pm 16(0.001)}{286 \pm 5(0.001)}$
20 **day**	$\frac{420 \pm 3.4}{100}$	$\frac{511 \pm 1.2(0.001)}{122 \pm 1.0(0.001)}$	$\frac{1110 \pm 10}{100}$	$\frac{1172 \pm 15(0.001)}{106 \pm 2(0.01)}$

1 month	$\frac{430 \pm 3.6}{100}$	$\frac{564 \pm 6.0(0.001)}{131 \pm 2.0(0.001)}$	$\frac{1120 \pm 11}{100}$	$\frac{1136 \pm 6(0.1)}{101 \pm 1(0.1)}$
2 month	$\frac{420 \pm 3.4}{100}$	$\frac{490 \pm 1.3(0.001)}{117 \pm 1.0(0.001)}$	$\frac{1130 \pm 16}{100}$	$\frac{1160 \pm 10(0.1)}{103 \pm 2(0.1)}$

With the simultaneous action of hypokinesia and radiation (4Grey), lipolytic activity does not change, blood activity increases lipolytic activity for 1 month, lowers it to the level of control values for 2 months.

The effect of hypokinesia and gamma irradiation increases the content of total pancreatic protein, and decreases it in the blood.Consequently, with the combined effect of these factors on synthesis, enzyme proteins and specific blood proteins are extraordinary.These factors enhance the synthesis of enzyme proteins, and reduce the synthesis of specific blood proteins.

With the simultaneous action of hypokinesia and radiation (4Grey), a decrease in the secretion of pancreatic enzymes may be the result of a weakening of stimulating effects at the level of their generation, as well as signals in the chain of neurons of the meta–sympathetic ganglia of the gland, as well as the result of inhibition of neurohumoral regulation processes, expressed in a violation of the balance of adrenergic and cholinergic mediation in the gastrointestinal tract, the predominance of destructive processes and microcirculation disorders, imbalance of hormones, and mediators. A decrease in the activity of pancreatic enzymes may also be the result of a violation of enzyme protein synthesis.

Thus, two periods are outlined in the development of functional changes in the animal body during experimental gamma irradiation. The initial one, when the changes characteristic of the stress reaction prevail, and the subsequent one, when violations of the synthesis of the protein molecule of enzymes in the pancreas are detected, the control and consistency of various metabolic links are disrupted.

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