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PROTEINS OXIDATIVE MODIFICATION AND MITOCHONDRIAL ENZYMES ACTIVITY IN RATS OF DIFFERENT AGES UNDER AFFECTION BY SODIUM NITRITES AND TOBACCO SMOKE

Topicality. Smoking continues to be one of the most important medical and social problems of modern society. The detrimental effect of smoking is associated with the presence of a large number of tobacco carcinogens and toxic substances. The widespread use of nitrates and nitrites in industry, agriculture led to pollution inorganic oxides of nitrogen and their chronic toxic effects on the human body. The study of combined influence of several xenobiotics in the body is appropriate and relevant.

The **aim** of the work – to investigate the performance of oxidative modification of proteins and activity of bioenergetic processes in rats of different ages for the defeat of sodium nitrite in 45 days on the background of toxicity by tobacco smoke.

Materials and methods. Experiments were conducted on rats of different ages, who for 45 days had been exposed to tobacco smoke. For 24 hours before the end of the experiment the animals were injected with sodium nitrite at a dose of 45 mg/kg of body weight, another group of sodium nitrite was administered 72 hours before euthanasia. Rats were taken out of the experiment under thiopental anesthesia. Serum and organs of animals were tested for 2,4-dynitrofenilhidrazone in organs – the activity of succinate dehydrogenase and cytochrome oxidase.

Results and discussion. On an experiment on rats of different ages we found that under the conditions of sodium nitrite poisoning on the background of 45 day toxicity by tobacco smoke there is activation of oxidative modification of proteins, as indicated by the increase of 2,4 dynitrofenilhidrazone serum and organs of animals. At this time we marked an inhibition of mitochondrial enzymes, indicating abuse bioenergetic processes in the body.

Conclusions. We found that the most pronounced changes in the processes of oxidative modification of proteins and bioenergy provision in the body of sodium nitrite affected against the background of tobacco intoxication were observed in immature and senile rats.

Key words: smoke; sodium nitrite; 2,4- dynitrofenilhidrazone; energy processes

П. Г. Лихацький, Л. С. Фіра, У. М. Федорович Процеси окисної модифікації білків та активність мітохондріальних ферментів у щурів різного віку за ураження натрію нітритом та тютюновим димом

Актуальність. Однією з найважливіших медико-соціальних проблем сучасного суспільства продовжує залишатись тютюнопаління. Згубний вплив паління пов'язаний з наявністю в тютюні великої кількості канцерогенних і токсичних речовин. Широке використання нітратів та нітритів у промисловості, сільському господарстві призвело до забруднення довкілля неорганічними оксидами азоту і їх хронічної токсичної дії на організм. Дослідження поєданого впливу декількох ксенобіотиків на організм є доцільним та актуальним.

Мета роботи – дослідити показники окисної модифікації білків та активність біоенергетичних процесів в організмі щурів різного віку за ураження натрію нітритом на тлі 45-денної інтоксикації тютюновим димом.

Матеріали та методи. Експерименти проведені на щурах різного віку, які протягом 45 днів піддавались впливові тютюнового диму. За 24 год до закінчення експерименту тваринам вводили натрію нітрит у дозі 45 мг/кг маси тіла, іншій групі тварин натрію нітрит вводили за 72 год до евтаназії. Щурів виводили з експерименту під тиопенталовим наркозом. У сироватці крові та органах тварин визначали вміст 2,4-динітрофенілгідразонів, в органах – активність сукцинатдегідрогенази та цитохромоксидази.

Результати та їх обговорення. В експерименті на щурах різного віку встановлено, що за умов отруєння натрію нітритом на тлі 45-денної інтоксикації тютюновим димом відбувається активація процесів окисної модифікації білків, на що вказує збільшення вмісту 2,4-динітрофенілгідразонів у сироватці крові та органах тварин. У цей час відмічено пригнічення активності мітохондріальних ферментів, що свідчить про порушення біоенергетичних процесів в організмі.

Висновки. Встановлено, що найбільш виражені зміни процесів окисної модифікації білків та біоенергетичного забезпечення в організмі, ураженому натрію нітритом на тлі тютюнової інтоксикації, відмічаються у ставонезрілих та старих щурів.

Ключові слова: тютюновий дим; натрію нітрит; 2,4-динітрофенілгідразони; енергетичні процеси

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Процессы окислительной модификации белков и активность митохондриальных ферментов у крыс разного возраста при поражении натрия нитритом и табачным дымом

Актуальность. Одной из важнейших медико-социальных проблем современного общества продолжает оставаться табакокурение. Пагубное влияние курения связано с наличием в табаке большого количества канцерогенных и токсических веществ. Широкое использование нитратов и нитритов в промышленности, сельском хозяйстве привело к загрязнению окружающей среды неорганическими окислами азота и их хроническим токсическим действием на организм. Исследование сочетанного воздействия нескольких ксенобиотиков на организм целесообразно и актуально.

Цель работы – исследовать показатели окислительной модификации белков и активность биоэнергетических процессов в организме крыс разного возраста при поражении натрия нитритом на фоне 45-дневной интоксикации табачным дымом

Материалы и методы. Эксперименты проведены на крысах разного возраста, которые в течение 45 дней подвергались воздействию табачного дыма. За 24 ч до окончания эксперимента животным вводили натрия нитрит в дозе 45 мг/кг массы тела, другой группе животных натрия нитрит вводили за 72 ч до эвтаназии. Крыс выводили из эксперимента под тиопенталовым наркозом. В сыворотке крови и органах животных определяли содержание 2,4-динитрофенилгидразонов, в органах – активность сукцинатдегидрогеназы и цитохромоксидазы.

Результаты и их обсуждение. В эксперименте на крысах разного возраста установлено, что при отравлении натрия нитритом на фоне 45-дневной интоксикации табачным дымом происходит активация процессов окислительной модификации белков, на что указывает увеличение содержания 2,4-динитрофенилгидразонов в сыворотке крови и органах животных. Отмечено подавление активности митохондриальных ферментов, что свидетельствует о нарушении биоэнергетических процессов в организме.

Выводы. Установлено, что наиболее выраженные изменения процессов окислительной модификации белков и биоэнергетического обеспечения в организме, пораженном натрия нитритом на фоне табачной интоксикации, отмечаются у неполовозрелых и старых крыс.

Ключевые слова: табачный дым; натрия нитрит; 2,4-динитрофенилгидразоны; энергетические процессы

INTRODUCTION

Background Tobacco smoking is undeniable today that only 14 % of the population in developed countries can prevent passive smoking, the latter routinely inhale smoke in an amount equivalent to an average of one scorched cigarette. In literature there is evidence that not only active smoking, but also contamination of indoor tobacco smoke is a risk factor in causing various diseases [1, 2]. Tobacco smoke contains a significant amount of free radicals, which penetrated inhaled into the airways, violate the balance of oxidants in the system of antioxidants. Free radical oxidation can be activated in adverse environmental conditions, under the influence of alcohol, tobacco smoke [3-8]. Excessive activation of lipid peroxidation leads to violation of the structure of membranes, lipid metabolism, has toxic effects on tissues, enhances lysis, oxidation of sulfhydryl groups of proteins and leads to the development of structural changes in the cardiovascular system, lungs, gastrointestinal tract [1, 9-11].

The interest in specification the mechanisms of the body to nitrites and nitrates and pathogenesis hemic hypoxia, which in this case occurs due to their wide use in industry, agriculture and medicine [12, 13].

Regardless the reasons that caused the occurrence of hypoxia, at a certain stage of its development there are universal violations in the form of lower production macroergic compounds violation ion homeostasis in the cell with the accumulation of calcium ions, increase free radical oxidation of phospholipids of cell membranes and subcellular [14].

In real life, people are often exposed to more toxic factors leading to the general poisoning of the body and in the process involving damage of many organs.

In the literature, there are not any research results of free and bioenergetic processes in animals under conditions combined influence of nitrites and tobacco smoke, which caused interest in connection with the spread of these toxins in the environment [15-18].

The **aim** of the work study was to examine the performance of proteins and oxidative modification activity bioenergetic processes in rats of different ages for the defeat of sodium nitrite in 45 day background toxicity of tobacco smoke.

MATERIALS AND METHODS

The experiments were conducted on white outbred male rats, which were kept on a vivarium standard diet of the Ternopil State Medical University. The rats were divided into three age categories – first – immature, weight – 60-80 g, the second – mature – weight – 180-200 g and the third – senile, whose body weight was 300-320 g. Each age group consists of two subgroups – intact control (C) and experimental group (E). Rats of experimental groups were exposed with tobacco smoke during 45 days. The model of the depending chronic smoke was created by means of airtight chamber volume of 30 liters that allows animals to fumigate free behavior. Tobacco smoke formed by burning of 6 cigarettes “Prima sribna (synia)” (containing 0.6 mg of nicotine and 8 mg of tar), was fed into it through openings in the chamber. Six animals were simultaneously in the chamber during 6 minutes. Ani-

mals in the control group were also 6 minutes in a sealed chamber, but were not subjected to smoke.

After 45 days from the beginning of the defeat of animals tobacco smoke deduced from the experiment by euthanasia under thiopental anesthesia.

For the study we took blood serum, liver, lungs and the myocardium of animals. From experimental tissue homogenates were prepared in 10 % isotonic solution.

Content product oxidative modification of proteins (2,4-dinitrofenilhidrazone (2,4-DNFH)) measured in the serum and tissue homogenates reaction with 2,4 dinitrofenilhidrazone method [19], the activity of succinate dehydrogenase [20] and cytochrome oxidase [21] measured in the liver, heart and lungs of animals.

We used the general principles of animal experiments in the research, approved at the National Congress on Bioethics (Kyiv, Ukraine, 2001) and consistent with the provisions of the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, France, 1985) [22, 23]. There were used parametric (according to Student) and

non-parametric (according to Wilcoxon) methods for data statistical analysis. Changes were considered as probable at $p \leq 0.05$ [24-26].

RESULTS AND DISCUSSION

The development of most pathological conditions occurs according to free radical mechanism that at the cellular level is characterized by increased production of free radicals, among which a special place belongs to reactive oxygen and nitrogen species (ROS/NS) [27-29]. Almost all cellular components are affected by ROS. Their interaction with proteins may lead to modifications of amino acid residues – oxidation of sulfhydryl groups of cysteine and methionine, histidine imidazole groups, cyclic rings tyrosine, phenylalanine, tryptophane [4]. These data are consistent with our research.

In the experiment, we found that after 45 days of affection of the rats of all ages tobacco smoke in the bodies of animals significantly ($p \leq 0.05$) increased the content of products of oxidative modification of proteins (2,4-DNFH) as the main (Tab. 1) and neutral (Fig. 1).

Table 1

THE CONTENT OF 2,4 DINITROPHENYLHYDRAZONE OF THE MAIN CHARACTER IN BLOOD SERUM (mmol/g PROTEIN) AND BODIES (mmol/g PROTEIN) IN RATS OF DIFFERENT AGES AFFECTED BY SODIUM NITRITE, AGAINST THE BACKGROUND OF 45 DAY TOXICITY BY TOBACCO SMOKE ($M \pm m$; $n = 72$)

Term study, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
blood serum			
intact rats	0.013 ± 0.001	0.010 ± 0.001	0.012 ± 0.001
45 day defeat by tobacco smoke	0.049 ± 0.001*	0.028 ± 0.001*	0.046 ± 0.003*
45 day defeat by tobacco smoke + 24 hours sodium nitrite poisoning	0.055 ± 0.001*	0.038 ± 0.001*	0.055 ± 0.002*
45 day defeat by tobacco smoke + 72 hours sodium nitrite poisoning	0.068 ± 0.001*	0.045 ± 0.002*	0.065 ± 0.004*
Liver			
intact rats	0.042 ± 0.001	0.026 ± 0.001	0.041 ± 0.001
45 day defeat by tobacco smoke	0.071 ± 0.002*	0.049 ± 0.001*	0.061 ± 0.003*
45 day defeat by tobacco smoke + 24 hours sodium nitrite poisoning	0.080 ± 0.003*	0.064 ± 0.003*	0.080 ± 0.005*
45 day defeat by tobacco smoke + 72 hours sodium nitrite poisoning	0.091 ± 0.005*	0.074 ± 0.004	0.083 ± 0.006*
Lungs			
intact rats	0.030 ± 0.001	0.019 ± 0.001	0.027 ± 0.002
45 day defeat tobacco smoke	0.054 ± 0.001*	0.036 ± 0.003*	0.042 ± 0.003*
45 day defeat by tobacco smoke + 24 hours sodium nitrite poisoning	0.060 ± 0.003*	0.038 ± 0.002*	0.049 ± 0.003*
45 day defeat by tobacco smoke + 72 hours sodium nitrite poisoning	0.069 ± 0.003*	0.047 ± 0.002*	0.052 ± 0.004*
Myocardium			
intact rats	0.028 ± 0.002	0.020 ± 0.002	0.025 ± 0.001
45 day defeat by tobacco smoke	0.047 ± 0.003	0.034 ± 0.002*	0.047 ± 0.002*
45 day defeat by tobacco smoke + 24 hours sodium nitrite poisoning	0.061 ± 0.002*	0.043 ± 0.001*	0.050 ± 0.002*
45 day defeat by tobacco smoke + 72 hours sodium nitrite poisoning	0.069 ± 0.002*	0.049 ± 0.003*	0.066 ± 0.002*

Note: here and in the following tables * – significant changes between intact rats and rats affected by tobacco smoke ($p \leq 0.05$).

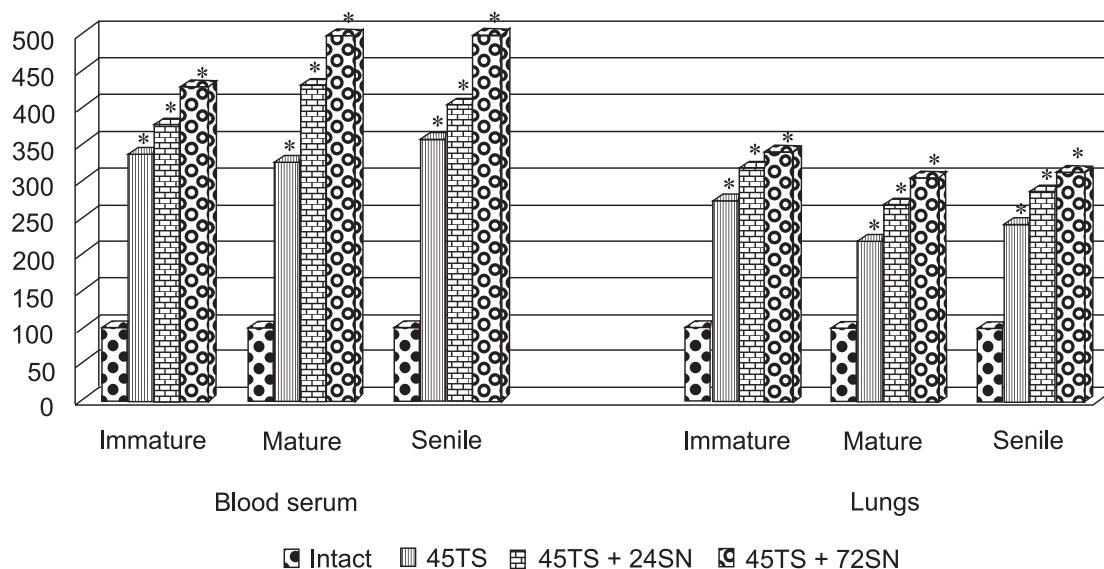


Fig. 1. Content of 2,4-dinitrophenylhydrazine neutral in the serum and lungs of rats infected with sodium nitrite in the background 45 day toxicity of tobacco smoke, %

Serum of immature rats after defeat by tobacco smoke the content of 2,4-DNFH of the main character grew in 3.1 times, in mature – in 2.8 times, in senile – in 3.8 times. Poisoning of rats with sodium nitrite led to an even more pronounced increase of products of oxidative modification of proteins. After 72 hours after poisoning toxins present content 2,4-DNFH exceeds the norm in serum immature animals in 5.2 times to mature – 4.5 times, in senile in 5.4 times.

A similar increase of oxidation modification products in the liver was noted in all age groups of rats after smoke intoxication. Most pronounced it was in mature animals and exceeded the level of intact rats in 1.9 times. At the end of the experiment on rats lesions additional toxicants sodium nitrite (72 hours on the background of 45 day toxicity of tobacco smoke) content of 2,4-DNFH immature animals exceeds the norm by 2.2 times to 2.8 times mature and in senile – in 2 times.

We studied the content oxidation modification products of the main character in the lungs of rats after defeat toxicants and noted that tobacco smoke intoxication is accompanied by increased content of 2,4-DNFH in immature animals on 80 %, in mature – on 89 % and in senile – on 55 %. Joining the additional influence of sodium nitrite to rats poisoning led to even more pronounced increase in this indicator immature lungs of animals on 130 % to 147 % in mature and senile – to 92 %.

The most sensitive to tobacco smoke was myocardium in senile rats, in which the content oxidation modification products 1.9 times higher than the level of intact animals. While the figure of immature and mature animals exceeds the rate of 1.7 times. Applying toxicants as an additional sodium nitrite deepened the degree of modification of protein molecules in the myocardium of animals. The least significant changes observed in the

myocardium of mature animals, where the content of 2,4-DNFH main character exceeded the rate in 2 times, in immature and senile rats this index increased in 2.5 and 2.6 times respectively.

Learning content of 2,4-DNFH neutral after the defeat of toxicants showed similar changes in all organs studied. The most pronounced increase of this indicator was observed in serum and lung lesions after experimental rats with both toxicants (Fig. 1).

So, after the defeat of rats with tobacco smoke the content of products oxidative modification of proteins increases in the studied organs, which undergoes further increase after administration of sodium nitrite poisoning animals. The most significant change in this indicator observed in senile animals.

The next stage of our research was to study the activity of mitochondrial oxidation processes in rats in conditions of simultaneous affection of tobacco smoke and sodium nitrite.

The most important function of mitochondria is intermediate oxidation metabolites carbohydrate, lipid and protein metabolism, including pyruvate, fatty acids, acetoacetate, etc., and the energy that is released when the decay of these compounds for the biosynthesis of ATP [30, 31]. Mitochondrial dysfunction associated with the processes of oxidative phosphorylation, mitochondrial structural integrity and identity of their genetic information system, arising under conditions of oxidative stress in diseases caused by metabolic disorders, and carcinogenesis [32, 33]. The main marker enzymes involved in oxidative phosphorylation violation is succinate dehydrogenase (SDG) and cytochrome oxidase (CO) [33-36].

When tobacco smoke toxicity in the liver, lungs and the myocardium of rats reduced the activity of SDG (Tab. 2).

Table 2

**ACTIVITY OF SUCCINATE DEHYDROGENASE IN THE LIVER, LUNGS AND
THE MYOCARDIUM (mmol/kg h) RATS OF ALL AGES AFFECTED BY SODIUM NITRITE
ON THE BACKGROUND OF 45 DAY TOBACCO INTOXICATION (M ± m; n = 72)**

Term of study, days	Groups of experimental animals		
	immature rats	mature rats	senile rats
Liver			
intact rats	33.66 ± 1.20	38.00 ± 1.15	34.66 ± 0.99
45 day defeat by tobacco smoke	18.33 ± 0.56*	21.67 ± 0.61*	20.16 ± 0.65*
45 day defeat by tobacco smoke + 24 hours sodium nitrite poisoning	18.00 ± 0.36*	20.00 ± 0.44*	19.00 ± 0.26*
45 day defeat by tobacco smoke + 72 hours sodium nitrite poisoning	15.50 ± 0.50*	18.67 ± 0.42*	17.67 ± 0.49*
Lungs			
intact rats	28.00 ± 0.73	31.00 ± 0.86	28.67 ± 0.67
45 day defeat by tobacco smoke	15.83 ± 0.79*	22.66 ± 0.84*	19.17 ± 0.31*
45 day defeat by tobacco smoke + 24 hours sodium nitrite poisoning	14.83 ± 0.75*	19.83 ± 0.48*	17.33 ± 0.80*
45 day defeat by tobacco smoke + 72 hours sodium nitrite poisoning	13.50 ± 0.62*	16.50 ± 0.56*	16.50 ± 0.34*
Myocardium			
intact rats	36.00 ± 0.73	41.66 ± 0.61	37.66 ± 0.61
45 day defeat tobacco smoke	22.33 ± 0.61*	27.66 ± 0.95*	23.00 ± 0.86*
45 day defeat tobacco smoke + 24 hours sodium nitrite poisoning	19.17 ± 0.31*	21.00 ± 0.82*	20.00 ± 0.68*
45 day defeat tobacco smoke + 72 hours sodium nitrite poisoning	17.67 ± 0.49*	19.33 ± 0.33*	18.17 ± 0.54*

Note: here and in the following tables * – significant changes between intact rats and rats affected by tobacco smoke ($p \leq 0.05$).

It is noted that lesions in rats by tobacco smoke almost equally affects the activity of SDG in the liver of animals of all ages (it decreases after 45 day toxicity in 1.7-1.8 times).

After entering the body poisoning smoke rats sodium nitrite found a marked reduction in the activity of the enzyme. And, the most sensitive to the action of the additional liver toxicants were immature rats, which SDG activity decreased in 2.3 times. In two other groups of animals, this indicator decreased by 2.0 times. In the study of enzyme activity in the lung we noted that the specified index suffered the biggest decline in the group of immature rats (decreased in 1.8 times, while in mature and senile – in 1.4 and 1.5 times, respectively).

In the myocardium of rats after intoxication by tobacco smoke SDG activity decreased in all age groups equally (in 1.5-1.6 times), was observed after the introduction of the affected body sodium nitrite. The activity of the enzyme appeared to be investigated in 2.0 times less intact control group of rats of all ages 72 hours after poisoning with sodium nitrite on the background of 45 day toxicity by tobacco smoke.

The results showed that under the defeat by tobacco smoke and sodium nitrite in the mitochondria of liver cells, heart and lungs disrupted energy balance of these cells, succinate as an important catalyst for energy metabolism in the body.

We investigated the activity of cytochrome oxidase, an enzyme which is a vector inner membrane of mitochondria and plays a key role in regulating the rate of oxidative phosphorylation. This enzyme is very sensitive to toxicants of different nature. This excessive adjustability is due to the fact that the vector enzymes perform important functions and have large supply adaptive capacity.

Immature and senile rats which were the most sensitive to the action of smoke and sodium nitrite lungs had the cytochrome oxidase activity after the defeat of both toxicants decreased by 61 % and 60 % respectively (at the end of the experiment) (Fig. 2).

Defeat by tobacco smoke resulted in a significant reduction ($p \leq 0.05$) cytochrome oxidase activity in rat's liver, which appeared on 57 % of immature, mature – on 84 % and 71 % in senile animals compared with intact control. Poisoning of rats with sodium nitrite further suppressed the activity of the enzyme, which is 72 hours after its use declined to 37 % in immature animals, 73 % in mature and to 53 % in senile.

In rats, poisoning by tobacco smoke, cytochrome oxidase activity in myocardium decreased almost equally in all age groups with a difference of 6.8 % in immature and senile compared to mature. After using sodium nitrite to hit lowest recorded cytochrome oxidase activity in myocardium senile animals (45 % of normal). In immature and mature rats the activity of the enzyme in the

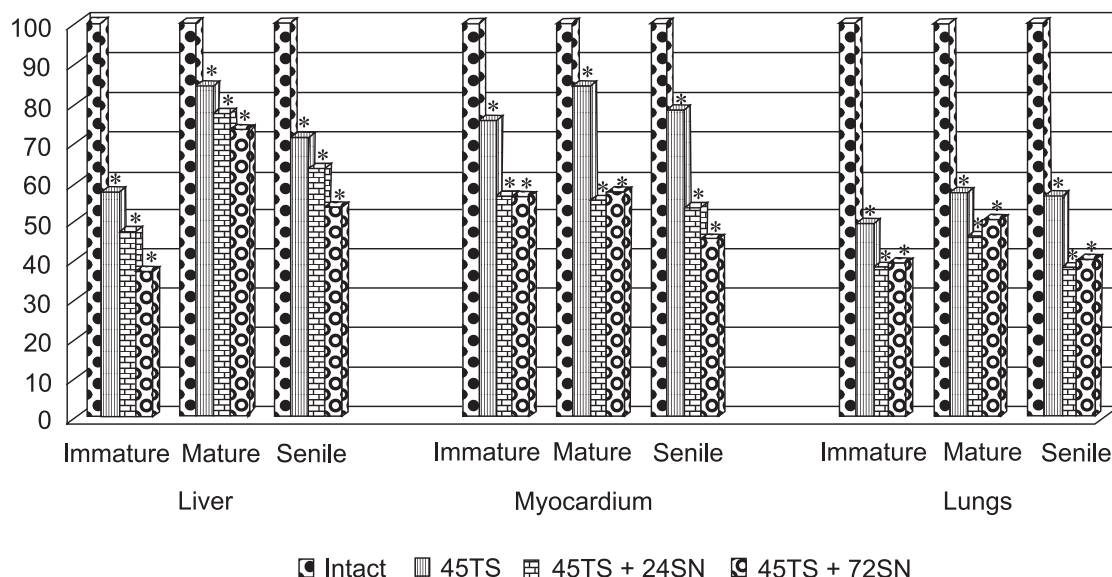


Fig. 2. Activity of cytochrome oxidase in the liver, lungs and the myocardium of rats of different age groups after the defeat of sodium nitrite intoxication on the background of tobacco, %

myocardium after the defeat of both toxicants (45 days of intoxication TS lesions and 72 h SN) was on 57 %.

So, lesions in rats with sodium nitrite intoxication on the background of tobacco smoke leads to a pronounced reduction in the activity of enzymes that converts substrates in the respiratory chain to form ATP energy.

CONCLUSIONS

In experiments on rats of different age groups we found that lesions of tobacco smoke leads to activation modification of proteins that is confirmed by increasing content of 2.4 dinitrofenilhidrazone basic and neutral in all organs. Injection of additional sodium nitrite toxicity factor further complicates oxidation-destructive processes in ani-

mals after injury. Content of product oxidative modification of proteins in organs increases linearly with the extension of the term of sodium nitrite. Along with the activation of destructive processes in the affected body it is marked the decrease in activity of the mitochondrial enzyme – succinate dehydrogenase and cytochrome oxidase, which are the key in regulating the formation of energy in the body. In the lungs, myocardium and liver of rats poisoned by sodium nitrite on the background of tobacco intoxication the activity of these enzymes was suppressed expressively.

The data showed that more sensitive to both toxicants were immature and senile animals.

Conflict of Interests: authors have no conflict of interests to declare.

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