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A.Ya.Velyka,

M.K.Bratenko

Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi

CORRELATION OF LIPID PEROXIDATION' PRODUCTS AND ANTIOXIDANT SYSTEM ENZYMES OF RATS' KIDNEY TISSUES IN CONDITIONS OF SALT LOADING AND EXPERIMENTAL NEPHROPATHY

Keywords: *salt loading, sublimate, thiobarbiturate-reaction products, glutathione-S-transferase, catalase, kidneys.*

Abstract. *The processes of lipid peroxidation in the tissues of rats' kidneys in case of 0.75% salt loading in conditions of mercury dichloride intoxication were studied in white nonlinear male rats. It was found that salt loading on the background of sublimate nephropathy leads to increase of thiobarbiturate-reaction products in different layers of kidneys in comparison with control. Increase of lipid peroxidation products caused disruption of pro-/antioxidant balance.That's why we studied activity of catalase and glutathione-S-transferase in rats' kidneys 72 hours after injection of mercury dichloride solution in the dose of 5 mg per 1 kg of animal's body weight, which is important for determination of mercury salts influence on the renal antioxidant system. The decrease of catalase activity in the renal cortex, medulla and papilla in case of salt loading after influence of mercury dichloride was found. For instance, loading with 0.75% solution of NaCl leads to the twofold increase of glutathione-S-transferase activity in comparison with control. Animals' intoxication with sublimate caused glutathione-S-transferase activity increase by 43% in the renal cortex and twofold - in the renal medulla in comparison with control. These results indicate the inhibition of antioxidant protection enzymes in the rats' kidneys in case of mercury dichloride influence.*
Pathogenetic unity of biochemical processes in the studied areas of the kidney is confirmed by the conducted regression analysis that proves interdependence of lipid peroxidation products and the system of antioxidant protection.

Introduction

Influence of xenobiotics, toxic compounds, and medicinal products on the organism leads to activation of the process of free radical oxidation of membranes. Lipid peroxidation activation induces significant changes of cellular metabolism and biomembrane functions, and is an important link of pathogenesis of many diseases, including those of kidneys [1, 3].

Action of toxic compounds of different nature causes infringement of kidneys cell membrane integrity and activation of macromolecules' free radical oxidation [2, 10].

It is known from literature sources [8] and was shown by us before [14] that any stress factor for the animal organism causes changes of antioxidant enzymes activity in the rats' kidneys.

Activation of free radical lipid and biopolymer peroxidation with hyperproduction of oxygen active forms, often on the background of organism antioxidant protective system exhaustion is considered to be the driving mechanism of cytolysis in case of any pathology.

This is evidenced by the results of numerous studies of liver [6] and kidneys glutathione system function abnormality in case of their acute and chronic diseases that promote formation of significant quantity of oxygen active forms and expression of their toxicity.

Kidneys are the main organ that regulates water-salt metabolism in the human organism and shows high selectivity to changes of water and salts excretion; that's why they are very sensitive to the influence of toxic compounds and in particular to the ions of heavy metals that accumulate in the kidneys [9]. When salts of mercury enter the body, 50% of its quantity is accumulated in the kidneys. It is worth noting that there are two forms of mercury fixation in the kidneys: labile part of the ion that determines the level of its excretion with the urine due to the secretory activity of the cells, and the inactive form that determines gradual accumulation of the element[13].

It was shown by us before [11] that in case of 3% salt loading in conditions of HgCl2 intoxication, the processes of lipids' free radical oxidation are activated in the rats' kidneys. Processes of antioxidant protection play an important role in pathogenesis of different diseases, because emergence of imbalance between activation of macromolecules' free radical oxidation and failure of antioxidant protection system can speed up the development of different pathological processes that are the basis of the renal diseases.

That's why it would be quite interesting to research the processes of lipid peroxidation and the status of the antioxidant system of the rats' kidneys in conditions of salt loading on the background of mercury dichloride intoxication.

The purpose of the study

To determine the changes of thiobarbiturate-reaction products content in the rats' kidneys tissues; activity of enzymes of glutathione S-transferase and catalase activities in the renal cortex, medulla, and papilla in conditions of 0.75% salt loading under the action of mercury dichloride; to determine the correlation between the products of lipid peroxidation and the antioxidant protection system of the rats' kidneys tissues.

Material and methods

The animals were kept in vivarium conditions with the constant temperature regime and free access to food and water. They were divided into groups: The 1st group (n=6) - the control group (intact animals that did nor receive loading); the 2nd group (n=6) - animals that had 0.75% salt loading (injection of 0,75% NaCl solution calculated as 0.65 mmoles of Na (14.8 mg of Na) for 100 g of the animal's body weight); the 3d group (n=6) - animals that received 0.1% sublimate solution subcutaneously and 72 hours after intoxication they received 0.75% salt loading. Loading was performed by intragastric injection through a metallic probe. 2 hours after the loading the animals underwent euthanasia through decapitation under light ether anesthesia. The experiments were conducted in accordance with the requirements of the European Convention on the Protection of Animals used for scientific purposes (86/609 EEC). After decapitation the kidneys were swiftly removed, thoroughly dried with filter paper and divided into layers: renal cortex, medulla, and papilla. A sample of renal layers (500 mg) was homogenized in 50 mM tris-HCl buffer (pH 7.4) that contained 0.1% of EDTA solution and the mixture was centrifuged for 10 minutes at 900G. All operations were performed only at the temperatures not higher than +4° C. The post-nuclear supernatants of renal layers were used to determine: 1. TBA-reaction products (TBA-RP) content by the reaction between malonaldehyde (derivatives) and thiobarbituric acid (TBA); in conditions of high temperature and acidic medium (pH 3.0) this reaction produces a pink trimethine complex. Absorption of the colored solution was measured with photoelectrocolorimeter КФК-3 (KFK-3) at wavelength of 532 nm. The TBA-RP content was expressed in μmoles/g of tissue [15]. 2. Catalase activity [EC 1.11.1.6] was determined by the reaction between not destroyed hydrogen peroxide and ammonium molybdate. Absorption of the colored complex was measured at wavelength of 410 nm. Enzyme's activity in the kidneys' supernatant was expressed in μmoles per minute for 1 g of tissue [7]. 3. Activity of Glutathione-S-transferase activity (G-S-T) [EC 2.5.1.18]. The method is based on spectrophotometric measurement of quantity of reduced glutathione conjugate with 1-chloro-2,4-dinitrobenzene; this conjugate forms under the action of the enzyme. Optical density of the produced complex was determined during the next 3 minutes using the spectrophotometer СФ-46 (SF-46) at the wavelength of 346 nm and expressed in nmoles of conjugate for 1 minute per 1 mg of protein [5].

Intoxication of animals with sublimate was conducted through subcutaneous injection of mercury (II) chloride water solution in the dose of 5 mg per kg of animal's body weight [4].

Discussion of the research results

Salt loading causes the change of the indexes of macromolecules' free radical oxidation in different layers of kidneys.

We determined that in case of 0.75% salt loading modeling in the rats' kidneys the content of TBA-RP increased in the renal cortex by 55%, in the renal papilla - by 58.4%, and in the renal medulla - by 45.7% in comparison with the control (fig. 1).

Intoxication of animals with 0.1% solution of sublimate in the dosage of 5 mg/kg of the animal's body weight led to the change of lipids free radical oxidation products indexes (fig. 2).

For instance, we found that 0.75% salt loading causes increase of TBA-RP content indexes in comparison with the control by 74% in the renal cortex, by 80% in the renal medulla, and in 2.5 times in the renal papilla in accordance with control.

From the antioxidant protection system we studied the changes of glutathione-S-transferase and catalase enzymes activity.

Glutathione-S-transferase (G-S-T) is an enzyme with polyfunctional activity that takes part in detoxification of certain xenobiotics, including peroxides. G-

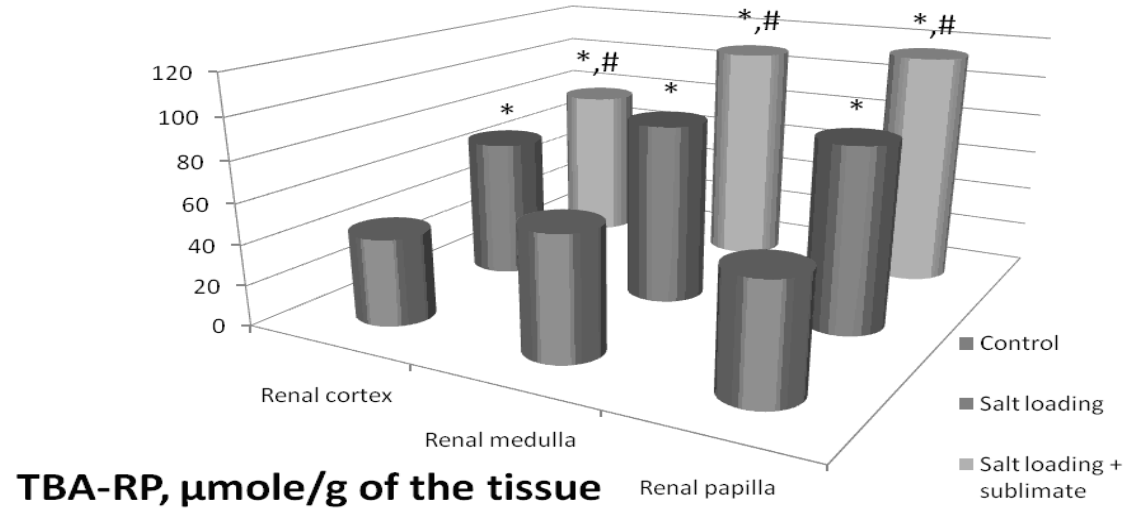

Figure 1. Thiobarbiturate-reaction products content in the rats' kidneys in case of 0.75% salt loading on the background of sublimate nephropathy

Here and on the figures 2 and 3 * the probable changes in comparison with the index of the animals from the control group (P<0.05) are shown; # - probable changes in comparison with the index of the salt loading (P<0.05).

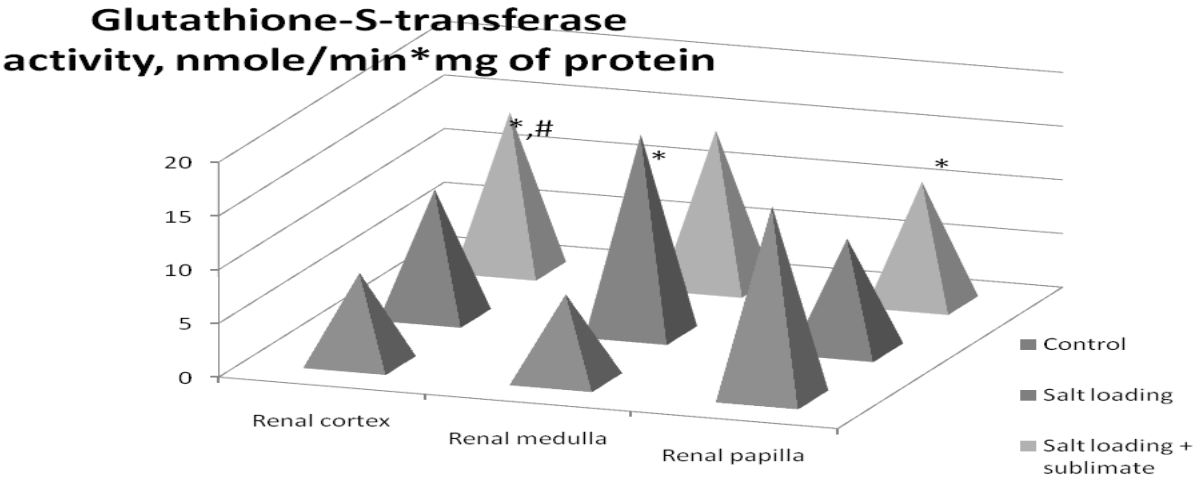

Figure 2. Glutathione-S-transferase activity in the rats' kidneys in case of 0.75% salt loading on the background of sublimate nephropathy

S-T catalyzes 4 main types of reactions; reduction of organic peroxides (hydroperoxides of fatty acids and cumene) to corresponding alcohols is one of them [12].

For instance, we found that 0.75% lading with NaCl solution leads to increase of glutathione-S-transferase activity in 2 times in comparison with control for which the value is 7.9 nmoles/min/mg of protein. In the renal papilla glutathione-S-transferase activity turned out to be lower than control by 40%, and it didn't change in the renal cortex.

Sublimate intoxication of animals led to the change of glutathione-S-transferase activity in different renal layers of rats. The studies revealed that 0.75% salt loading caused glutathione-S-transferase activity increase by 43% in the renal cortex and twofold - in the renal medulla in comparison with control. But in conditions of sublimate intoxication and 0.75% salt loading the same index decreased for renal papilla by 47% in comparison with control values.

Catalase is an enzyme of antioxidant system that can take part in restoring of pro-/antioxidant balance when external factors affect the body.

In the renal medulla in conditions of 0.75% salt loading twofold decrease of catalase activity in comparison with control was noted.

In the rats' renal papilla 0.75% loading with NaCl solution led to the decrease of catalase activity by 75% in comparison with the control (fig. 3).

In conditions of HgCl2 intoxication adaptation of antioxidant renal system to the action of this prooxidant takes place.

Pathogenetic unity of biochemical and physiological processes in the studied areas of the kidney is confirmed by the conducted regression analysis that proves interdependence of lipid peroxidation products and the system of antioxidant protection.

This data is confirmed by multifactorial regres-

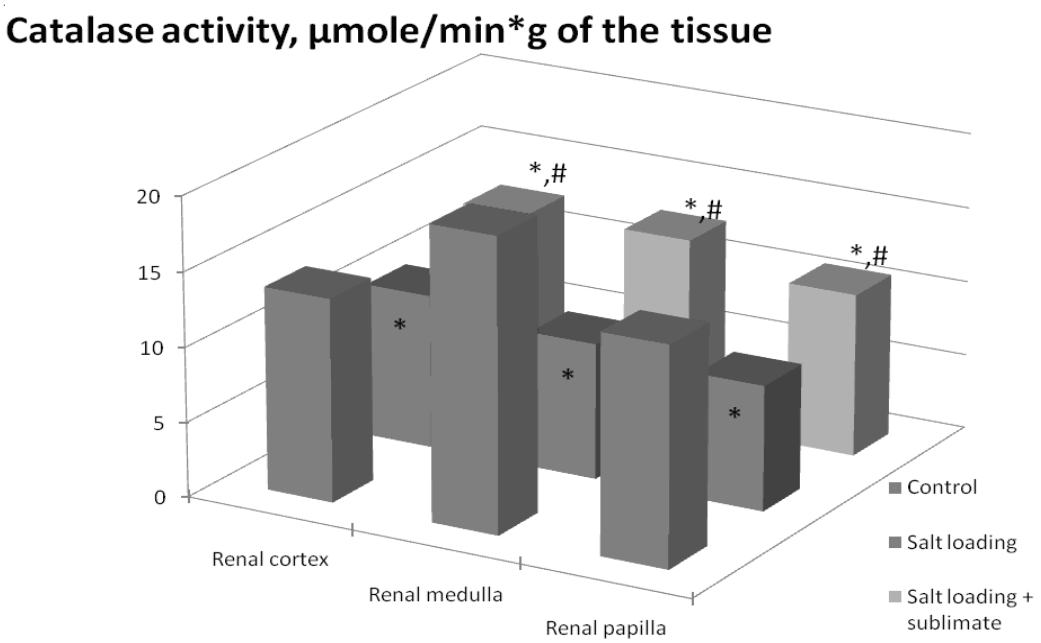

Figure 3. Catalase activity in the rats' kidneys in case of 0.75% salt loading on the background of sublimate nephropathy

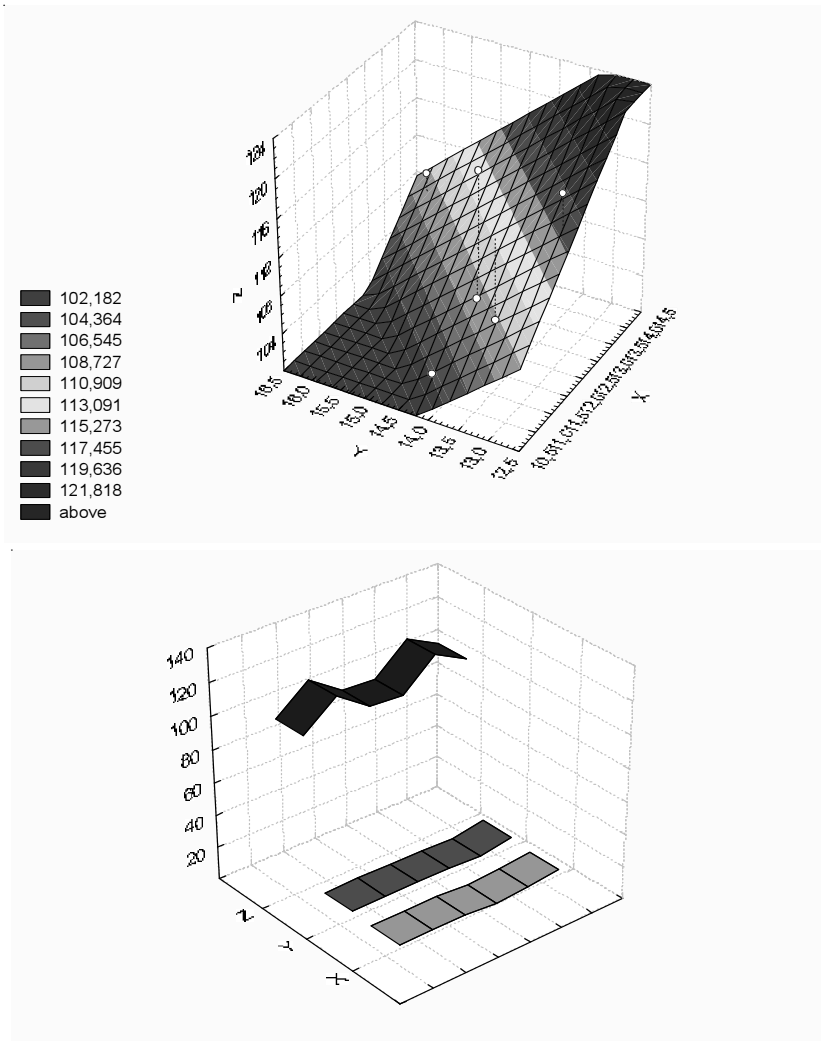

Figure 4. The diagram of multifactorial regression analysis of reliable connections ($p< 0.05$) between catalase activity (X - µmole/min * g of the tissue), malonaldehyde content (Z - µmole/g of the tissue), glutathione-S-transferase activity (Y - nmole/min * mg of protein) in the renal medulla in case of sublimate nephropathy in sexually mature rats in conditions of loading with 0.75% sodium chloride solution

sion analysis of reliable connections between catalase activity, thiobarbiturate-reaction products content, and glutathione-S-transferase activity in the renal medulla in case of sublimate nephropathy in rats in conditions of salt loading with 0.75% sodium chloride solution (fig. 4). The intensity of the shading corresponds to the degree of correlation.

Therefore rats' intoxication with mercury dichloride solution leads to the destruction of the cell membrane and causes activation of macromolecules' free radical oxidation process. In turn this stimulates the antioxidant system of the animal's organism, which takes part in neutralization of oxygen active forms.

Conclusions

1.Subcutaneous injection of 0.1% mercury dichloride solution in the dose of 5 mg/kg of the animal's body weight in conditions of 0.75% loading leads to suppression of catalase activity

2.Pro-/antioxidant balance under the influence of toxin is supported by increase of glutathione-S-transferase

3.Intoxication of animals with 0.1% solution of sublimate in the dosage of 5 mg/kg of the animal's body weight led to the change of lipids free radical oxidation products indexes

4.It was found that 0.75% salt loading causes increase of TBA-RP content indexes in comparison with the control by 74% in the renal cortex, by 80% in the renal medulla, and in 2.5 times in the renal papilla

5.Pathogenetic unity of biochemical and physiological processes in the studied areas of the kidney is confirmed by the conducted regression analysis that proves interdependence of lipid peroxidation products and the system of antioxidant protection.

Prospects for further studies

In the future we plan to study the influence of salt loading on the functional condition of kidneys in case of experimental nephropathy.

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КОРЕЛЯЦІЯ ПРОДУКТІВ ПЕРЕКИСНОГО ОКИСНЕННЯ ЛІПІДІВ ТА ЕНЗИМІВ АНТИОКСИДАНТНОЇ СИСТЕМИ ТКАНИН НИРОК ЩУРІВ ЗА УМОВ СОЛЬОВОГО НАВАНТАЖЕННЯ Й ЕКСПЕРИМЕНТАЛЬНОЇ НЕФРОПАТІЇ

А. Я. Велика, М. К. Братенко

Резюме. На білих нелінійних щурах-самцях було досліджено процеси пероксидного окиснення ліпідів в тканинах нирок щурів за умов 0,75% сольового навантаження при інтоксикації меркурію дихлоридом. З'ясовано, що сольове навантаження на тлі сулемової нефропатії призводить до зростання вмісту тіобарбітурат-реакційних продуктів у порівнянні з контролем в різних шарах нирок. Зростання продуктів перекисного окиснення ліпідів призвело до порушення про-/антиоксидантного балансу.

Тому вивчали активність каталази, глутатіонтрансферази у нирках щурів через 72 години після введення розчину меркурію дихлориду в дозі 5 мг на кг маси тіла тварин, що є важливим для з'ясування впливу солей меркурію на антиоксидантну систему нирок. Встановлено, зниження активності каталази у кірковій, мозковій речовині та сосочку нирок за умов сольового навантаження після дії меркурію дихлориду. Так, навантаження 0,75% розчином натрію хлориду (NaCI) призводить до зростання активності глутатіонтрансферази у два рази порівняно з контролем. Інтоксикація тварин сулемою призвела до зростання глутатіонтрансферазної активності у порівнянні з контролем на 43% - у кірковому шарі нирок та вдвічі - у мозковому. Отримані результати свідчать про пригнічення ферментів антиоксидантного захисту у нирках щурів за дії меркурію дихлориду.

Патогенетична єдність біохімічних процесів в досліджуваних ділянках нирки підтверджується проведеним регресійним аналізом, що підтверджує взаємозалежність продуктів перекисного окиснення ліпідів та системи антиоксидантного захисту.

Ключові слова: сольове навантаження, сулема, тіобарбітурат-реакційні продукти, глутатіонтрансфераза, каталаза, нирки.

КОРРЕЛЯЦИЯ ПРОДУКТОВ ПЕРОКСИДНОГО ОКИСЛЕНИЯ ЛИПИДОВ И ЭНЗИМОВ АНТИОКСИДАНТНОЙ СИСТЕМЫ ТКАНЕЙ ПОЧЕК КРЫС В УСЛОВИЯХ СОЛЕВОЙ НАГРУЗКИ И ЭКСПЕРИМЕНТАЛЬНОЙ НЕФРОПАТИИ

А. Я. Великая, М. К. Братенко

Резюме. На белых нелинейных крысах-самцах исследовали процессы пероксидного окисления липидов в тканях почек крыс в условиях 0,75% солевой нагрузки при интоксикации меркурия дихлоридом. Выяснено, что солевая

нагрузка на фоне сулемовой нефропатии приводит к возрастанию содержания тиобарбитурат-реакционных продуктов в сравнении с контролем в разных слоях почек. Увеличение продуктов пероксидного окисления липидов привело к нарушению про-/антиоксидантного баланса.

Поэтому изучали активность каталазы, глутатионтрансферазы в почках крыс через 72 часа после введения раствора меркурия дихлорида в дозе 5 мг на кг массы тела животных, что является важным для выяснения влияния солей меркурия на антиоксидантную систему почек. Установлено, снижение активности каталазы в корковом, мозговом веществе и сосочке почек в условиях солевой нагрузки после действия меркурия дихлорида. Так, нагрузка 0,75% раствором натрия хлорида (NaCl) приводит до увеличения активности глутатионтрансферазы в два раза в сравнении с контролем. Интоксикация животных сулемой привела к возростанию глутатионтрансферазной активности в сравнении с контролем на 43% - в корковом слое почек и в два раза - в мозговом. Полученные результаты свидетельствуют про угнетение ферментов антиоксидантной защиты в почках крыс при действии меркурия дихлорида.

Патогенетическое единство биохимических процессов в исследованных участках почки подтверждается регрессионным анализом, что подтверждает взаимозависимость продуктов пероксидного окисления липидов и системы антиоксидантной защиты.

Ключевые слова: солевая нагрузка, сулема, тиобарбитурат-реакционные продукты, глутатионтрансфераза, каталаза, почки.

Высшее государственное учебное заведение "Буковинский государственный медицинский университет", г. Черновцы

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