European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 03, 2020

NFLUENCE OF INDUCTION AND INHIBITION OF THE MONOOXYGENASE SYSTEM OF THE LIVER ON THE THYROID STATUS OF THE RAT'S BODY

[1]Yuldashev N.M., [2]Rasulova M.T., [1]Abzalova Sh.R.

[1] Tashkent Pediatric Medical Institute, Republic of Uzbekistan
[2] Fergana branch of the Tashkent Medical Academy Republic of Uzbekistan

e-mail: y_nosir@rambler.ru
e-mail: abzalova71@mail.ru

Abstract: In order to assess the dependence of the thyroid status of the organism on the functional state of the monooxygenase system of the endoplasmic reticulum of hepatocytes in sexually mature male rats, induction and inhibition of the monooxygenase system of the liver by its inductors and inhibitors was caused. As inducers, we used known induction drugs - benzonal (benzobarbital) and zixorin (flumecinol), and as inhibitors a chemical compound - cobalt chloride and a blocker of histamine H2-receptors - cimetidine. Benzonal was administered orally at a dose of 50 mg / kg body weight for 3 days, and zixorin was administered at a dose of 40 mg / kg body weight for 4 days. Cobalt chloride was administered once intraperitoneally at a dose of 30 mg / kg of body weight, and cimetidine was administered orally at a dose of 100 mg / kg for 10 days. It was revealed that during the induction of the monooxygenase system of the liver by its inductors - benzonal and zixorin, there is an increase in the content of thyroid hormones - triiodothyronine and thyroxine in the blood against the background of no change in the level of thyroid-stimulating hormone. When the monooxygenase system of the liver is inhibited by its inhibitors - cobalt chloride and cimetidine, an increase in the content of thyroid hormones - triiodothyronine and thyroxine in the blood is also observed, but against the background of a statistically significant decrease in the content of thyroid stimulating hormone. It is concluded that there is an indirect relationship between the functional state of the monooxygenase system of the liver and the thyroid status of the body.

Keywords: thyroid status, monooxygenase system, induction, inhibition, benzonal, zixorin, cobalt chloride, cimetidine.

Introduction. Thyroid hormones - thyroxine (T4) and triiodothyronine (T3) are essential regulatory molecules for the development, growth and functioning of cells and organs. They regulate the level of basal cell metabolism, including hepatocytes. Therefore, a change in the level of thyroid hormones should affect the functioning of the liver. At the same time, deiodination of thyroid hormones occurs in the liver with the help of enzymes-deiodinases. Therefore, the functional activity of hepatocytes can also affect the content of thyroid hormones. It is known that T3 has a greater affinity for nuclear receptors than T4. At the same time, T4 is secreted 10 times more than T3. Deiodination of T4 to T3 occurs with the help of 3 groups of enzymes-deiodinases: D1, D2 and D3. These enzymes are responsible for both the conversion of T4 to the more active T3 and the inactivation of T4 by converting to reverse T3 and converting reverse T3 and T3 to deiodothyronine. The conversion of T4 to T3 in tissues occurs under the action of type D1 deiodinases, which is found mainly in the liver and kidneys. Deiodinase D3 plays the main role in the inactivation of both T4 and T3. It is localized in the liver, skin, and central nervous system. In addition to participating in metabolism, a number of plasma proteins that bind thyroid hormones are synthesized in the liver: thyroxine-binding globulin, thyroxine-binding prealbumin and albumin. In most chronic diseases, thyroid hormone metabolism disorders are observed, characterized by a normal level of total T4, a normal or increased level of free T4 (cT4), a low level of total T3, a low level of free T3 (cT3) and an increased level of reverse T3. This condition is called sick euthyroid syndrome. Revealing the relationship between the thyroid status of the body and the functioning of the liver will allow in the future to develop methods to regulate the functioning of both the liver itself and the thyroid gland.

Purpose of the study. Evaluation of the dependence of the thyroid status of the organism on the activity of the microsomal monooxygenase system of the liver in the experiment in rats.

Material and methods. The experiments were carried out on 70 white male rats weighing 180-220 g. The animals were kept under standard vivarium conditions with natural light and free access to water and food. The experimental protocol was in accordance with the ethical standards set out in the "Rules for working with experimental animals", as well as in Directive 2010/63 / EU of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes.

Induction of the monooxygenase system of the liver in 40 rats was caused by its inductors - benzonal (benzobarbital) and zixorin (flumecinol). In 15 rats, induction of the monooxygenase system of the liver was carried out by oral administration of benzonal on starch paste for 3 days at a dose of 50 mg / kg body weight. In 15 rats, induction of the monooxygenase system of the liver was carried out by oral administration of zixorin on starch paste for 4 days at a dose of 40 mg / kg body weight. Inhibition of the monooxygenase system of the liver in 40 rats was caused by its inhibitors - cobalt chloride (CoCl2) and cimetidine. In 15 rats, the monooxygenase system of the liver was inhibited by a single intraperitoneal injection of CoCl2 at a dose of 30 mg / kg body weight. In 15 rats, the monooxygenase system of the liver was inhibited by oral administration of cimetidine on starch paste for 10 days at a dose of 100 mg / kg body weight. 10 rats served as control.

To assess the metabolic activity of the liver monooxygenase system, a hexenal test was performed. Geksenal was administered to animals at a dose of 100 mg / kg, intraperitoneally. The time between the loss and the acquisition of the "flipping reflex" was taken into account. The animals were placed in a thermostatic chamber with a temperature of 26 ° C. After awakening, the rats were killed under ether anesthesia by decapitation and blood was collected. In the blood serum, the content of free and total triiodothyronine and thyroxine, thyroid stimulating hormone (TSH) was determined by the method of enzyme-linked immunosorbent assay ELISA, using the test systems of the company "Human" (Germany) on a microplate photometer MR96A (Mindray, China).

The rat liver was homogenized in a glass homogenizer with a Teflon pestle in an isolation medium consisting of 0.25 M sucrose, 0.05 M KCl in a solution of 0.05 M Tris-HCl buffer, pH = 7.4. The homogenate was subjected to differential centrifugation and the content and activity of the components of the monooxygenase system were determined in the microsomal fraction. The content of cytochrome P-450 in the microsomal suspension was determined by the method of T. Omura, R. Sato [15,17]. The content of cytochrome b5 was determined after reconstitution of the microsome suspension test samples with the addition of NADH. The rate of p-hydroxylation of aniline in the microsomal fraction was estimated by the formation of p-aminophenol, and N-demethylation of amidopyrine in the microsomal-cytosolic fraction by the formation of formaldehyde [2]. Protein content in samples was determined according to Lowry et.al.

The obtained digital results were processed using standard methods of variation statistics using the Student's t-test.

Research results. To identify the relationship between the thyroid status of the body and the functionally active state of the monooxygenase system of the liver, two inducers, benzonal and zixorin, were selected as inducers of this system.

Benzonal (1-benzoyl-5-ethyl-5-phenylbarbituric acid) is an inducer of phenobarbital type cytochrome P-450. Under its influence, the content of microsomal protein, cytochrome P-450 and the activity of NADPH cytochrome P-450 reductase significantly increases in the liver. Indeed, the results of studies have shown that in animals with benzonal induction of the monooxygenase system of the liver, the duration of hexenal sleep was shortened by 36.6% compared to the intact indicator (Table 1).

Table 1. Content and activity of microsomal components monooxygenase system of the liver during induction and inhibition

	Duration of hexenal sleep, min.	Microsomal cytochrome content, nmol / mg protein		Activity of microsomal enzymes, nmol / min • mg protein	
		P-450	b5	Anilinide roxylase	Amidopyrine-N-demethylase
Control	28,00 ± 0,87	0,99 ± 0,09	0,41 ± 0,03	0,94 ± 0,08	2,79 ± 0,26
Benzonal	17,75 ± 0,75*	1,52 ± 0,13*	0,48 ± 0,03	1,29 ± 0,11*	4,22 ± 0,41*
Zixorin	22,13 ± 2,5*	1,39 ± 0,08*	0,50 ± 0,05	1,19 ± 0,04*	5,02 ± 0,48*
CoCl2	48,63±0,25*	0,44±0,03*	0,28±0,04*	0,53±0,08*	1,22±0,07*
Cimetidine	51,25±0,25*	0,40±0,06*	0,25±0,03*	0,39±0,04*	1,02±0,08*

Note: * - P <0.05 compared with intact values

The content of the main component of the monooxygenase system, cytochrome P-450, was increased by 53.5% of the intact value. Although the absolute value of cytochrome b5 was increased from the intact value by 17.1%, this difference from the control was not statistically significant (P> 0.05). Aniline hydroxylase and amidopyrine-N-demethylase activity of microsomes during benzonal induction was higher than intact values by 37.2 and 51.3%, respectively.

Zixorin is 3-fluoromethyl-α-ethylbenzhydrol in chemical structure. It also increases the content of components of the liver microsomal monooxygenase system. Under its influence, the formation of glucuronides and the secretion of bile are enhanced. In animals with induction of the monooxygenase system of the liver with zixorin, the duration of hexenal sleep was shortened by 21.0% compared to the intact value (see Table 1).

Zixorin induction also led to an increase in the content of cytochrome P-450. Thus, it turned out to be 40.4% higher than the intact value. In the content of cytochrome b5, similar

to benzonal induction, although an increase of 22.0% from the control was observed, however, this increase was not statistically significant. With zixorin induction, aniline hydroxylase activity of microsomes was 21.4% higher than intact values, and amidopyrine-N-demethylase activity was 79.9% higher.

Consequently, benzonal and zixorin lead to significant induction of the liver monooxygenase system.

To identify the relationship between the thyroid status of the body and the functionally depressed state of the monooxygenase system of the liver, two substances, CoCl2 and cimetidine, were chosen as inhibitors of this system.

The research results showed that in animals with inhibition of the monooxygenase system of the liver by CoCl2, the duration of hexenal sleep was extended by 73.7% compared to the intact indicator (see Table 1). At the same time, the content of the main component of the monooxygenase system, cytochrome P-450, was reduced by 55.6% of the intact value. The content of cytochrome b5 was reduced from the intact value by 31.7%. The aniline hydroxylase and amidopyrine-N-demethylase activity of microsomes upon inhibition of MOS was lower than intact values by 43.6 and 56.3%, respectively.

When cimetidine was administered to experimental animals, the duration of hexenal sleep was lengthened by 83.0% compared to the intact value (see Table 1). Cimetidine inhibition also led to a decrease in the content of cytochrome P-450. Thus, it turned out to be 59.6% lower than the intact value. The content of cytochrome b5 showed a 39.0% decrease from the intact value. With cimetidine inhibition of MOC, the aniline hydroxylase activity of microsomes turned out to be lower than intact values by 58.5%, and amidopyrine-N-demethylase - by 63.4%.

Consequently, cobalt chloride and cimetidine lead to significant inhibition of the liver monooxygenase system.

The study of the thyroid status during benzonal induction of the monooxygenase system of the liver showed a statistically significant increase in the blood content of total T3 by 23% and free by 11.7% of intact values (Fig. 1).

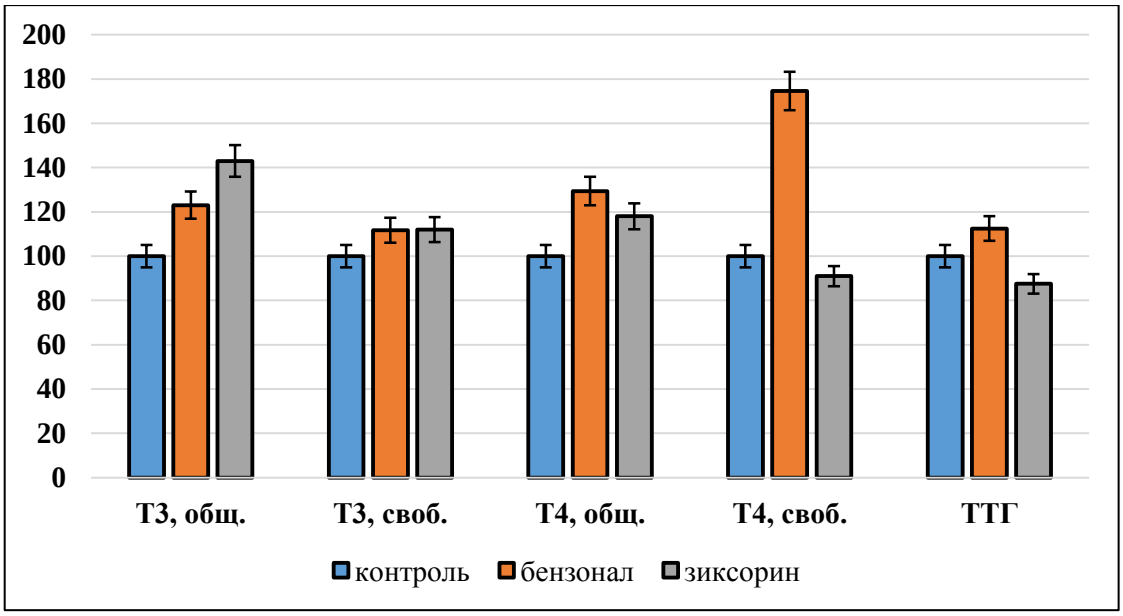


Figure: 1. Thyroid status of the body with benzonal and zixorin induction of the monooxygenase system of the liver.
The ordinate is the content of the indicators in%.

An increase was also observed in the T4 content. Thus, the content of total T4 was increased by 29.4%, and free - by 74.6% of the control. Although the absolute value of TSH was increased by 12.5% compared with the control, it was statistically insignificant.

The study of the thyroid status during zixorin induction of the monooxygenase system of the liver also showed a statistically significant increase in the blood content of total T3 by 43% and free - by 12.0% of intact values (see Fig. 1). The content of total T4 was increased by 18.0%, and in the content of free T4 and TSH, statistically significant changes from intact parameters were not revealed.

Thus, the results obtained showed that with the induction of the monooxygenase system of the liver by its inductors - benzonal and zixorin, an increase in the content of thyroid hormones - T3 and T4 in the blood is observed, against the background of practically no change in the level of TSH.

The study of the thyroid status during inhibition of the monooxygenase system of the liver with cobalt chloride showed a statistically significant increase in the blood content of free T3 by 39.3% of the intact value, against the background of no change in its total content (Fig. 2).

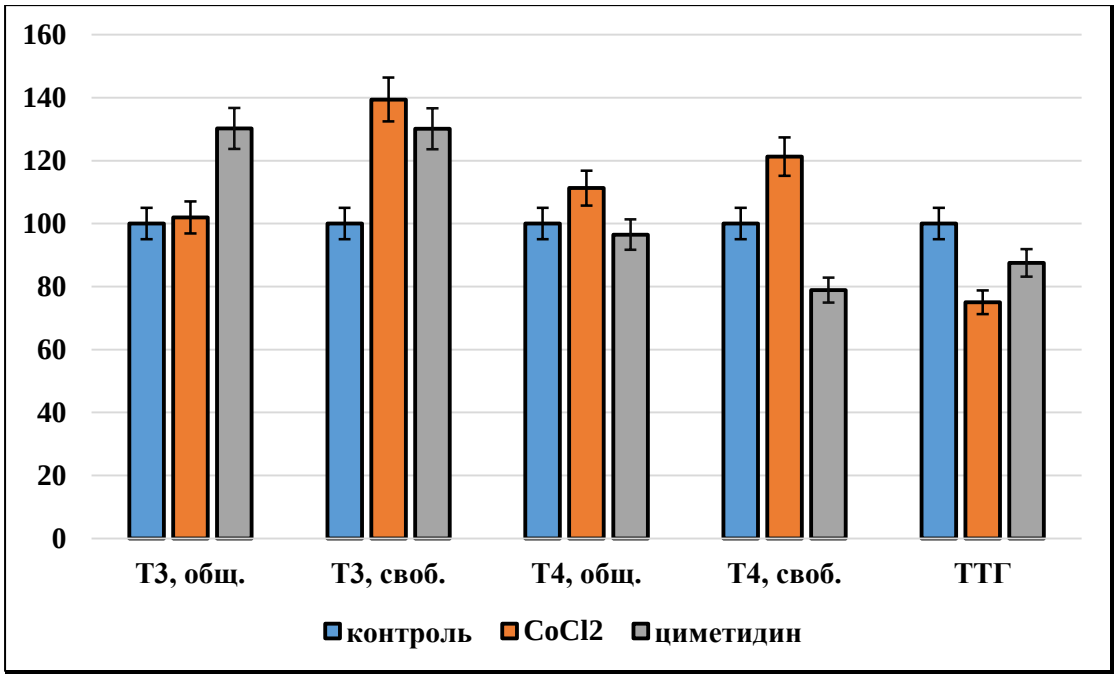


Figure: 2. The thyroid status of the body during inhibition of monooxygenase liver systems with cobalt chloride and cimetidine.
The ordinate is the content of the indicators in%

The increase in the content of total T4 by 11.3% of the intact value was not statistically significant (P> 0.05), however, the content of free T4 was higher than the control by 21.3%. In the content of TSH with inhibition of MOC by cobalt chloride, a statistically significant decrease from control by 25.0% was observed.

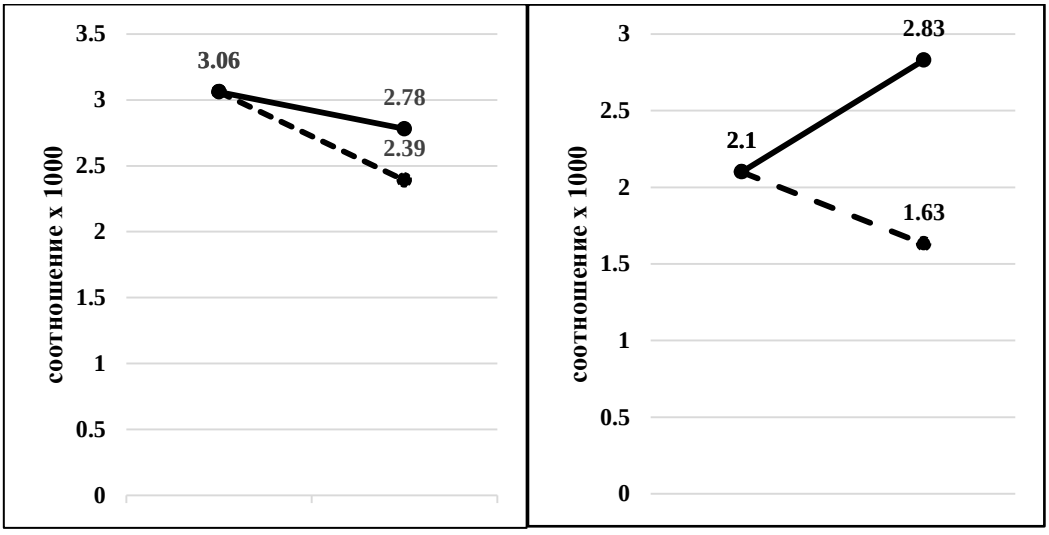
The study of the thyroid status during cimetidine inhibition of the monooxygenase system of the liver showed a statistically significant increase in both total and free T3 levels in blood by 30.2 and 30.1%, respectively, from intact values (see Fig. 2). No statistically significant change from the intact value was observed in the total T4 content. At the same time, the content of free T4 was reduced by 21.1%. The TSH content turned out to be statistically significantly reduced by 12.5% compared to the control.

Thus, the results obtained showed that when the monooxygenase system of the liver is inhibited by its inhibitors - cobalt chloride and cimetidine, there is a slight increase in the content of thyroid hormones - T3 and T4 in the blood, against the background of a decrease in the content of TSH.

Discussion. In the study, for the induction of the monooxygenase system of the liver, the "reference" inducers of microsomal oxidation - benzonal and zixorin - were used. Although both of them belong to the phenobarbital type of inducers [3, 4], they differ in the “spectrum” of inducible cytochrome P-450 isoforms. Benzonal induces cytochrome isoforms

P-450IIB, P-450IIC, P-4501IIA, and zixorin induces cytochrome P-450IA, P-450IIB isoforms. The introduction of benzonal to rats causes an increase in the content of cytochrome P-450 and the activity of NADPH cytochrome P-450 reductase, against the background of rapid activation of CYP2B1 and CYP2B2 transcription [13]. Under the influence of benzonal, protein synthesis is also quite strongly increased [5], which is also confirmed by morphological studies. Thus, benzonal induction, similar to phenobarbital induction, is manifested by an increase in the volume of hepatocytes due to an increase in the volume of their cytoplasm and, to a lesser extent, nuclei. So M.V. Zakharova showed that phenobarbital induction leads to an increase in the volume of hepatocytes, their cytoplasm and nuclei, respectively, by 74, 77 and 42.7% of control [1]. At the same time, in this work, it was shown that during zixorin induction, the volume of hepatocytes, cytoplasm and nuclei increased by 32, 33 and 27.3%, respectively, compared to the control. With the introduction of zixorin, unlike benzonal, there is no increase in protein synthesis [5].

This is probably the reason for the multidirectional changes in the content of thyroid hormones during benzonal and zixorin induction. To illustrate these changes, we analyzed the changes in the cT3 / T3 and cT4 / T4 ratios at different induction of the liver monooxygenase system. The results showed that, under benzonal induction, a decrease in the cT3 / T3 ratio and an increase in cT4 / T4 (Fig.3a) are observed, while with zixorin induction, both cT3 / T3 and cT4 / T4 decrease (Fig. 3b).



а б

Figure: 3. Change in the ratio of cT3 / T3 (a) and cT4 / T4 (b) at benzonal (solid line) and zixorin (dashed line) induction

One of the possible mechanisms of these changes may be the specific effect of the used inducers on the rate of protein synthesis. Benzonal induction also strongly induces protein synthesis. T3 and T4 in the blood circulate in thyroxine-binding globulin, thyroxine-binding prealbumin and albumin. At the same time, it is free T3 and T4 that influence all stages of metabolism, growth and development, maintaining body temperature and stimulating heat production. In our experiments with benzonal induction, a statistically significant increase in the content of both free T3 and free T4 was observed, which is probably the basis for the enhancement of biosynthetic processes leading to hypertrophy of the subcellular structures of hepatocytes and enhancement of protein synthesis.

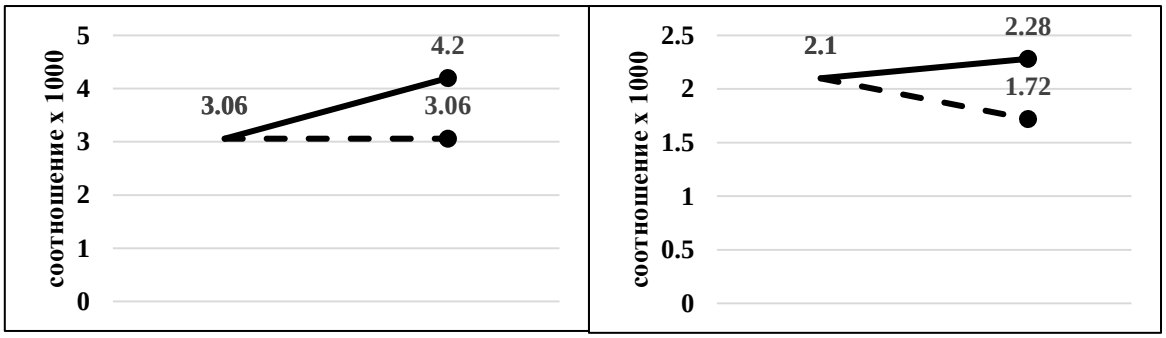
It is known that the synthesis, secretion and action of thyroid hormones are controlled by the hypothalamic-pituitary-thyroid system. Thyrotropin-releasing factor, secreted by the hypothalamus, stimulates the synthesis and secretion of thyroid-stimulating hormone. In our studies, it was with benzonal induction that there was a tendency to an increase in TSH levels. Zixorin induction was accompanied by an increase in the level of free T3 only. The content of free T4 even tended to decrease. At the same time, the TSH content had the same tendency.

In the study, the "reference" inhibitors of microsomal oxidation, cobalt chloride and cimetidine, were used to inhibit the monooxygenase system of the liver. Although both substances belong to the inhibitors of the monooxygenase system of the liver, they are completely different in chemical structure. Cobalt is an essential element for the normal functioning of the body, as it is part of a number of enzymes. However, high doses of this element are toxic [9, 14]. It exhibits genotoxicity [8, 12], causes oxidative stress [7, 16] and hypoxia [6]. At the same time, the molecular mechanisms of cobalt toxicity are not fully understood. The decrease in the content of microsomal cytochromes P-450 and b5 upon administration of cobalt salts is possibly associated with the induction of heme oxygenase [11]. Inactivation of cytochrome P-450 in cytochrome P-420 during intoxication with cobalt salts is also possible due to phosphorylation of its protein part [10, 18].

Cimetidine - N-cyano-N'-methyl-N "-guanidine. Its empirical formula is C10H16N6S. This substance is the first synthesized blocker of histamine H2-receptors, thereby inhibiting acid secretion in the stomach. Cimetidine inhibits NADPH-dependent microsomal oxidation, associated with cytochrome P-450, which leads to a decrease in the content of reduced cytochrome P-450 and a significant suppression of the aniline hydroxylase activity of liver monooxygenases.Probably, it is the differences in the inhibition mechanisms that were the reason for the multidirectional changes in the content of thyroid hormones during cobalt

chloride and cimetidine inhibition changes were analyzed ratios of cT3 / T3 and cT4 / T4 with different forms of inhibition of the monooxygenase system of the liver.

The results showed that with inhibition by cobalt chloride, an increase in the ratio of both cT3 / T3 and cT4 / T4 is observed (Fig.4a), while with cimetidine inhibition, there is no change in the cT3 / T3 ratio (P> 0.05) and a decrease in cT4 / T4 (Fig.4b).



а б

Figure: 4. Change in the ratio of cT3 / T3 (a) and cT4 / T4 (b) at chloride cobalt (solid line) and cimetidine (dashed line) inhibition

In both cases, the changes occurred against the background of a decrease in the TSH content. It is known that the TSH content is the first to respond to thyroid dysfunction. Its decrease can manifest itself in the asymptomatic stages of the disease, when the T3 and T4 indicators are still normal. In our experiments, against the background of an increase in the level of T3 and partly T4, a decrease in the level of TSH was observed and, in particular, when the monooxygenase system was inhibited by cobalt chloride. These changes probably indicate dysfunction of the thyroid gland due to the direct (maybe in the case of cobalt chloride inhibition) or indirect (maybe in the case of cimetidine inhibition) effect of the applied inhibitors on it.

Thus, the research results have shown that both induction and inhibition of the monooxygenase system leads to an increase in the content of thyroid hormones. However, with induction, there is not a change or a tendency to an increase in the level of TSH, but with inhibition, its statistically significant decrease. The results obtained indicate a relationship between the functional state of the monooxygenase system of the liver and the content of thyroid hormones in the body. However, some of the results can be explained in terms of the individual influence of the drugs used. Based on this, we can conclude that the relationship between the functional state of the monooxygenase system and the thyroid status is not direct, but mediated.

References

[1] Zakharova M.V. The influence of inducers of enzymes of monooxygenases on the processes of damage and recovery of the liver after ischemia: author. dis ... c.m.s. Novosibirsk, 1994.19 p.

[2] Lakin K.M., Krylov Yu.F. Biotransformation of medicinal substances. M .: Medicine, 1981.342 p.

[3] Lampatov V.V. The role of the liver and kidneys in the elimination of X-ray contrast agents (experimental study): dis ... Doctor of Biological Sciences. Barnaul, 2003.229 p.

[4] Novozheeva T.P., Smagina M.I., Cherevko N.A., Fateeva S.N. Benzobarbital and fluorobenzobarbital - inducers of the phenobarbital type of the monooxygenase system of the liver // Bulletin of Siberian Medicine. 2011. No. 5. P. 78-81.

[5] Novozheeva T.P., Chursina I.E., Novozheeva A.V., Saratikov A.S. The effect of benzonal, halothane and halodif on the antitoxic function of the liver of rats with extrahepatic cholestasis // Chemical-Pharmaceutical Journal. 2004. v. 38, No. 1. P. 3-4.

[6] Arizono K., Okanari E., Ueno K., Ariyoshi T. Heme oxygenase activity and cytochrome P-450 content associated with induced metallothionein in the liver of rats treated with various metals. J. Environ. Sci. Heals. 1991. vol. 26. no. 6. P. 941-951.

[7] Bruick R.K. Oxygen sensing in the hypoxic response pathway: regulation of the hypoxia-inducible transcription factor. Genes Dev. 2003. vol. 17. P. 2614-2623.

[8] De Boeck M., Kirsch-Volders M., Lison D. Cobalt and antimony: genotoxicity and carcinogenicity. Mutat. Res. 2003. vol. 533. P. 135-152.

[9] Edel J., Pozzi G., Sabbioni E. et al. Metabolic and toxicological studies on cobalt. Sci. Total Environ. 1994. vol. 150. P. 233-244.

[10] Elliason E., Mkrtchan S., Halpert J.R., Ingelmansundberg M. Substrat-regulated, cAMP-dependent phosphorilation, denaturation, and degradation of glucocorticoid-inducible rat liver cytochrome P-450 3A1. J. Biol. Chem. 1994. vol. 269. no. 28. P. 18378-18383.

[11] Jansson J., Curti M., Epstein P.M., et al. Relationship between phosphorilation and cytochrome P-450 destruction. Arch. Biochem. Biophys. 1990. vol. 283. no. 2. P. 285-292.

[12] Lison D., De Boeck M., Verougstraete V., Kirsch-Volders M. Update on the genotoxicity and carcinogenicity of cobalt compounds. Occup. Environ. Med. 2001. vol. 58. P. 619-625.

[13] Nelson D.R., Kamataki T., Waxman D.J. The P450 superfamily: update of new sequences, gene mapping, as session numbers, really trivial names, and nomenclature. DNA and Cell Biology. 1993. vol. 12. no. 1. P. 1-51.

[14] Nemery B., Lewis C.P.L., Demedts M. Cobalt and possible oxidant-mediated toxicity. Sci. Total Environ. 1994. vol. 150. P. 57-64.

[15] Omura T., Sato R. The Carbon Monoxide-binding Pigment of Liver Microsomes. I. Evidence for Its Hemoprotein Nature. J. Biol. Chem. 1964. vol. 239. P. 2370-2378.

[16] Abzalova, S., Primova, G., Kaldibaeva, A.. Using innovative methods in teaching pathological anatomy at a medical institute. Using innovative methods in teaching pathological anatomy at a medical institute// Journal of Advanced Research in Dynamical and Control Systems 12(2). 2020. P. 2770-277.

[17] Abzalova, Shakhnoza Rustamovna. "Morphofunctional changes in the liver in experimental ischemic brain damage." Young Scientist 10 (2015): 375-379.

[18] Rustamova, Abzalova Shakhnoza, Utekeeyeva Sanaubar Satullayevna, and Kaldibayeva Akmaral Orinbasarovna. "Characteristic morphologic alterations in liver in experimental ischemic damage of brain." *European science review* 7-8 (2015).

[19] Abzalova, Shakhnoza Rustamovna. "New aspects of teaching pathological anatomy" Academy 7 (46) (2019).

[20] Abzalova, Sh.R. Scientific study procedure / Abzalova, Sh.R. , Gulyamov, S.S., Ashurova, D.T., Husainov, J.B.// International Journal of Psychosocial Rehabilitation 24 (Special Issue 1), 2020, p. 928-936