# INFLUENCE OF THYROXIN ON TISSUE RESPIRATION IN LIVER OF RATS AFTER EXPERIMENTAL INTOXICATION BY HERBICIDE 2,4-DMA

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## Introduction

This work was undertaken as a part of research into disorders of thyroid-dependent metabolic reactions at intoxication by 2,4-D-dimethylamine salt (2,4-DMA), the effective herbicide still widely used in agriculture and manufactured in Ufa, Russia. We reported on thyroid hormonal status changes[1] and activity of some enzymes in liver homogenate of rats[2] in conditions of experimental intoxication by 2,4-DMA. The aim of the present study was to estimate the general intensity of tissue respiration in liver homogenate of rats at subchronic 4-week 2,4-DMA intoxication. We were also interested in the influence of exogenous thyroid hormones on these processes.

## Materials and Methods

The experiments were performed on 102 male rats of 180-230 grams body weight who received daily the 2,4-DMA water solution intragastrally during 4 weeks in a total dose equivalent to $LD_{50}$ and 1/10 $LD_{50}$. Control animals received sodium chloride solution. Upon the termination of the experiment the rats were decapitated and their liver collected. Both the thin (0.3 to 0.5 mm) layers of liver tissue and the mitochondrial suspension selected from liver homogenate were incubated in the presence of $^{14}C$-labeled oxidation substrates (1,4-$^{14}C$-succinate, 1,2-$^{14}C$-α-oxoglutarate and 2-$^{14}C$-pyruvate); $^{14}CO_2$ formed was caught by a paper moistened with NaOH solution. The activity of the paper was measured using beta-counter, and the results were expressed relatively to weigh of native tissue sample or mitochondrial protein content. As the intoxication by phenoxyherbicides is accompanied by reduction of plasma T4 level, at the end of intoxication period (48 hours before decapitation) the control and intoxicated animals of the separate groups were once intraperitoneally injected with the solution of L-thyroxin (Sigma, USA; 1 mg/100 g body weight). The intensity of tissue respiration in those animals was investigated the above mentioned way. All statistic data processing was performed using Student t-criterion.

## Results and Discussion

The results obtained testify about the changes of oxydative metabolism intensity both in liver tissue and separated mitochondria in conditions of 4-week 2,4-DMA intoxication. The pyruvate oxidative consumption in mitochondrial suspension was reduced (down to 51.8% of control level) at all doses of toxicant, whereas the succinate and α-oxoglutarate metabolism was slightly intensified at 1/10 $LD_{50}$ dose and significantly reduced (to 74.7% and 86.6%) at $LD_{50}$ dose of 2,4-DMA (Table 1).

Table 1. Intensity of $^{14}C$-labeled substrates oxidation in mitochondrial suspension from the liver of rats at intoxication by different doses of 2,4-DMA and after introducing of thyroxin (activity of caught $^{14}CO_2$; $*10^5$ pulses per min/mg of protein; M±m)

| Substrate | Control group | Control + thyroxin | 1/10 $LD_{50}$ 2,4-DMA | 1/10 $LD_{50}$ + thyroxin | $LD_{50}$ 2,4-DMA | $LD_{50}$ + thyroxin |
|---|---|---|---|---|---|---|
| α-Oxoglutarate | 3.96±0.58 | 2.06±0.09* | 4.05±0.87 | 7.91±1.56* | 2.83±0.55* | 3.51±0.50 |
| % of control | - | 52.0 | 102.2 | 199.9 | 71.6 | 88.6 |
| Succinate | 3.98±0.98 | 2.46±0.05* | 4.78±1.81 | 5.67±0.32* | 3.37±0.67 | 4.22±0.69 |
| % of control | | 61.7 | 119.9 | 142.1 | 84.5 | 105.8 |
| Pyruvate | 5.7_±1.50 | 2.97±0.12* | 4.37±0.41 | 4.59±1.32 | 2.96±0.59* | 6.67±2.23 |
| % of control | - | 51.9 | 76.5 | 80.4 | 51.8 | 116.7 |

* - statistically significant changes ($p<0.05$)

The same tendency was observed in thin layers of liver tissue: the induction of tissue respiration at 1/10 $LD_{50}$ dose of 2,4-DMA (except case of pyruvate) and its general depression at $LD_{50}$ (Table 2). These results testify about the original "irritating" effect concerning acceleration of specified substrates' oxidation (except pyruvate) in liver tissue and hepatocytes' mitochondria at small doses of toxicant. The purpose of it may be the energy supply of activated detoxication and plastic cell processes, including microsomal oxidation and conjugation. At the same time the decrease of pyruvate oxidation can be connected with especial vulnerability and/or sensivity of pyruvate dehydrogenase, or occurance of metabolic block on tricarbonic cycle initial reactions caused by toxicant.

Table 2. Intensity of $^{14}C$-labeled substrates oxidation in thin layers of liver of rats at intoxication by different doses of 2,4-DMA and after introducing of thyroxin (activity of caught $^{14}CO_2$; $*10^6$ pulses per min/g of tissue; M±m)

| Substrate | Control group | Control + thyroxin | 1/10 $LD_{50}$ 2,4-DMA | 1/10 $LD_{50}$ + thyroxin | $LD_{50}$ 2,4-DMA | $LD_{50}$ + thyroxin |
|---|---|---|---|---|---|---|
| α-Oxoglutarate | 3.59±0.27 | 4.08±0.32 | 3.67±0.15 | 5.15±0.55* | 2.63±0.27* | 3.20±0.37 |
| % of control | - | 113.5 | 102.1 | 143.4 | 73.3 | 89.1 |
| Succinate | 3.24±0.25 | 3.73±0.32 | 3.49±0.34 | 4.30±0.31 | 2.63±0.15 | 2.95±0.18 |
| % of control | - | 115.3 | 107.8 | 132.7 | 81.2 | 91.1 |
| Pyruvate | 5.47±0.35 | 6.24±0.32 | 4.47±0.30* | 5.39±0.24 | 3.61±0.41* | 5.29±0.28 |
| % of control | - | 114.0 | 81.8 | 98.5 | 66.0 | 96.8 |

* - statistically significant changes ($p<0.05$)

Higher dose of 2,4-DMA ($LD_{50}$) resulted in depression of oxidation of all substrates investigated. This effect is probably caused by significant destroy of mitochondrial membranes[6] and its enzymes by the herbicide itself and its metabolites as well as by products of activated peroxidation. Different metabolic blocks and accumulation of some metabolites may also occur, as it was described concerning succinate dehydrogenase and cytochrome C reductase in experiments with in vitro incubation of mitochondria in the presence of 2,4-DMA[3].

The shifts found out are capable to play a role in development of cell energy insufficiency at intoxication[3,4], especially concerning occurance of relative or absolute hypothyroid status[1]. The additional assignment of thyroxin in our experiments resulted in amplification of specified substrates' oxidative metabolism in all groups (liver tissue) and all groups except the control (mitochondrial suspension). At 1/10 $LD_{50}$ dose of 2,4-DMA its "irritating" action was probably added to inductive respiratory effect of thyroxin, and the total activity of succinate and α-oxoglutarate oxidation was increased 1.5-2 times to control level. At higher doses of toxicant the more significant effect of thyroxin was displayed (especially to pyruvate): the speed of substrates' oxidation was strongly elevated to near control level.

We're puzzle to explain the "reversed" effect of thyroxin in the control group of rats: the rate of substrates' oxidation was about 2 times lower to intact animals. This paradoxical reaction as a consequence of hyperthyreoidism and excessive activation of peroxidation was probably observed, resulting in damages to enzymes in mitochondrial membrane and/or depletion of enzymatic protein synthesis. Thus, the introduction of thyroxin at subchronic intoxication by 2,4-DMA in rats generally normalizes the oxidative utilization of specific substrates, and may be one of correcting factors of metabolic disorders caused by phenoxyherbicides.

**References**

1. Kamilov F., Davletov E., Gilmanov A. and Galimov Sh. (1997) Organohal. Comp. *34*, 178-181.
2. Gilmanov A., Abdullina G. and Kamilov F. (1999) Organohal. Comp. *42*, 317-320.
3. Palmeira C., Moreno A., Madeira V. (1994) Toxicol. Appl. Pharmacol. *127(1)*, 50-57.
4. Palmeira C., Moreno A., Madeira V. (1994) Arch.Toxicol. *68(1)*, 24-31.