Original article **UDC: 612.45-015.1: 577.164.1**
doi:10.5633/amm.2014.0206

THE STUDY OF VITAMINS B1, B6, AND B12 EFFECTS ON ADRENAL CORTEX ADAPTATION BY MONITORING SOME ENZYME SYSTEMS IN RATS TRAINED BY SWIMMING

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The adrenal hormones play a central role in response to environmental stimuli, both internal and external. We analyzed enzymes activities (LDH- lactate dehydrogenase, GLDH-glutamate dehydrogenase and AcPh – acid phosphatase) in adrenal cortex through swimming exercises and under the influence of B-group vitamins.

The analyzed cases in the experiment revealed significant increase of enzyme activities, namely in the zona fasciculata and zona reticularis of the adrenal cortex. Physical exertion is a form of stress and causes steroidogenesis process expression. The vitamins used take part as co-ferments in production of a lot of enzymes and in their activities as well.

Improvement of the enzyme system in adrenal glands in animals through swimming training with addition of vitamins B1, B6 and B12 leads to faster and long-term production of hormones necessary for stress response known as General Adaptation Syndrome. *Acta Medica Medianae 2014;53(2):33-39.*

Key words: *adrenal cortex, LDH, GLDH-glutamate dehydrogenase and AcPh enzymes, vitamins B1, B6 and B12*

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Introduction

Hans Selye defined the term "adaptation syndrome" as a group of symptoms that occur during acute and chronic stress exposure (1). Adaptation refers to adjustment of the cellular enzyme system, as well as of the morphology of the cells themselves, tissues and organs in response to alternations of internal and external environmental factors (2) in order to restore homeostasis (3,4). In other words, excessive physiological stresses, especially prolonged ones, or some pathological stimuli, may cause numerous cellular and morphological cell adaptations with new cellular complexes of altered state and location that preserve the vitality of cells and also modify their function in response to such stimuli (5). Adaptation syndrome is a complex cascade of events comprising the stress response (4) and causing the alterations in neuroendocrine and immune functions, levels of hormones, enzymes and in gastrointestinal functions (6). Stress and accompanying emotional distress are important risk factors for cardiovascular disorders (6). The disruption of cortisol circadial rhythm secretion is associated with stress, especially with secretion of melatonin and vitamin B12 (6). Such responses or reactions are "non-specific", that is, they occur regardless of the nature of stressors (1).

Adaptation process to prolonged stress shows systemic effects in the increase of stress hormone in plasma, such as cortisol and catecholamines (6,7).

Adrenal glands are vital organs under the constant influence of internal and external environmental factors. Among numerous stressogenic factors that affect adrenal gland, emotional and physical activities have been documented (8). Such activities affect adrenal glands, causing their morphological and biochemical alterations, as well as subtle relationship between enzymes, having a great impact on synthesis and secretion of this gland.

However, physical exertion undoubtedly modifies the cellular enzyme system in some organs, thus enabling alterations in stress-exposed cells. In order to modify and improve such an enzyme system in adrenal cortex, it is possible to "strengthen" it by the use of appropriate B-group vitamins that may function as co-enzymes (9-12).

Lactate - dehydrogenase (LDH) is an enzyme of the glycolytic pathway and its increase reflects cell damage. It is a protein enzyme belonging to pyridine dehydrogenase group and catalyzes the transfer of a hydride ion from the reduced nicotinamide adenine dinucleotide (NADH) and vice versa. Levy et al. proved expressed activity of this enzyme in adrenal cortex that significantly decreases in hypophysectomy (13).

Acid phosphatases (AcPh) are phosphatases that hydrolyze phosphate esters at Ph optimum less than 7.0. They can be found in lysosomes of the secretory epithelial cells. Although phosphatase is primarily produced by the prostate gland, it can also be found in erythrocytes, platelets, leukocytes, bone marrow, liver, spleen, kidneys and small intestine. Significant differences in adrenal acid phosphatase localization are present and depend on the type of an animal. The activity of this enzyme is dominant in the inner layer of the zona fasciculata and zona reticularis (14).

Glutamate-dehydrogenase is a mitochondrial enzyme that catalyzes oxidative deamination of glutamic acid to form α-ketoglutarate, yielding free ammonia.

Material and methods

The experiment was performed on albino rats weighing between 250-300 g. They were divided into four groups of 20 animals each.

The first group of animals underwent swimming training for twelve weeks in the pools with water of 299-303° K, duration time was from 6.102 -9.102 s.

The second, third and fourth group of animals were intraperitoneally given vitamins B1, B6 and B12 at a dose of 20i.u./kg body weight.

The presence of LDH-lactate dehydrogenase, GLDH-glutamate dehydrogenase and AcPh–acid phosphatase in adrenal cortex glands in rats was assessed using comparative histochemical methods by Pearse (15).

Aim

The aim of our study performed on rats which underwent swimming training for 12 weeks was to evaluate the presence of LDH-lactate dehydrogenase, GLDH-glutamate dehydrogenase and AcPh – acid phosphatase in adrenal cortex zones in rats, using comparative histochemical methods, and to establish their behavior under the influence of vitamins B1, B6 and B12.

Results

In the group of animals trained by swimming without vitamin treatment, a partial positive reaction of glutamate dehydrogenase was present in the zona glomerulosa and the zona reticularis, and almost negative in the zona fasciculata (Figure 1). Unlike the previous dehydrogenase, LDH was very positive in the zona glomerulosa and in the zona fasciculata (Figure 2) in the same group of animals, while the reaction to AcPh was negative in the zona fasciculate and in the zona reticularis, and weakly positive in the zona glomerulosa (Figure 3).

Figure 1.

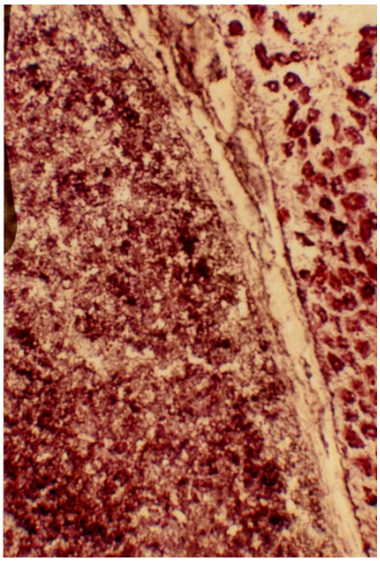

Figure 2.

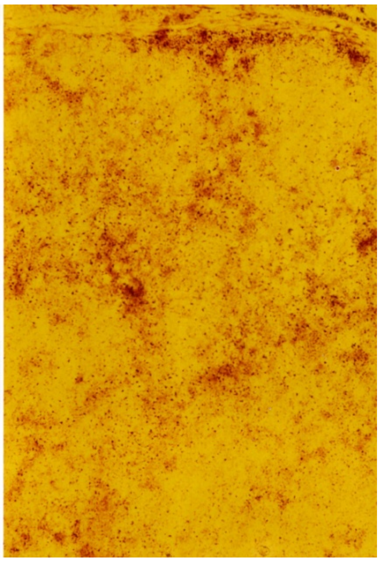

Figure 3.

First group of animals

The first group of animals trained through swimming without addition of vitamins.

Figure 1 - a partially positive reaction to glutamate dehydrogenase and almost negative in the zona fasciculata 120x

Figure 2 - very positive LDH in the adrenal zona glomerulosa and fasciculata in trained, but not treated animals, 120x

Figure 3 - negative reaction to AcPh in the zona fasciculata and zona reticularis, but weakly positive in the zona glomerulosa, 120x

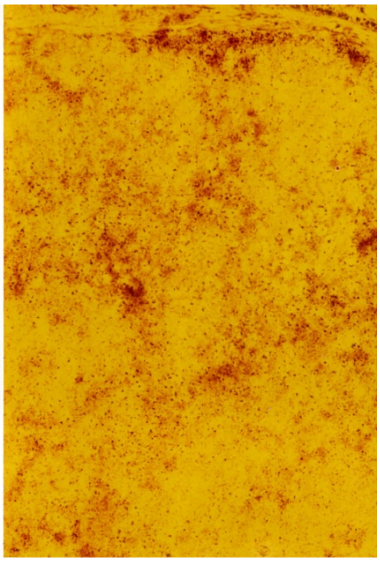 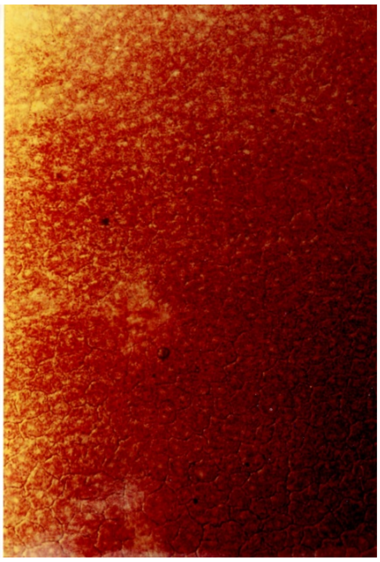 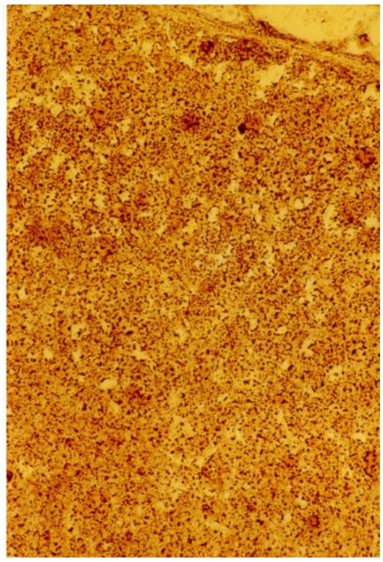

Figure 4. *Figure 5.* *Figure 6.*

Second group of animals

The second group of animals were given vitamin B1 prior the training.

Figure 4 – a weakly positive reaction to glutamate dehydrogenase in the zona glomerulosa and especially positive in the outer parts of the zona fasciculata, 120x
Figure 5 – positive reaction LDH in the zona glomerulosa, strongly positive in the zona fasciculata, especially in its outer part 120x
Figure 6 – AcPh is weakly positive in the zona glomerulosa, and negative in the suprarenal zona fasciculata, 120x

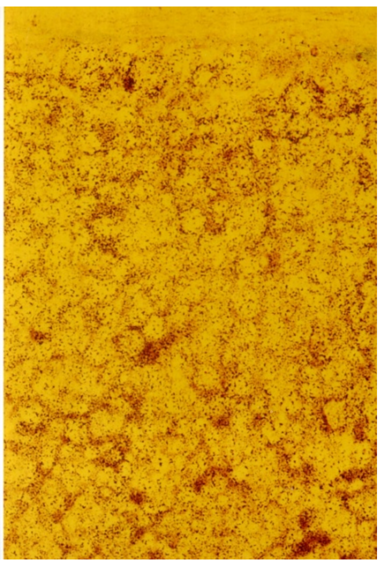 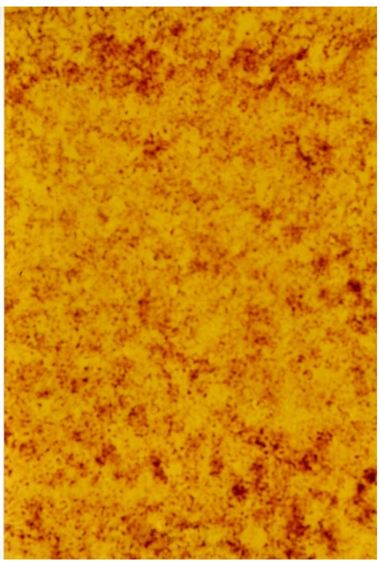 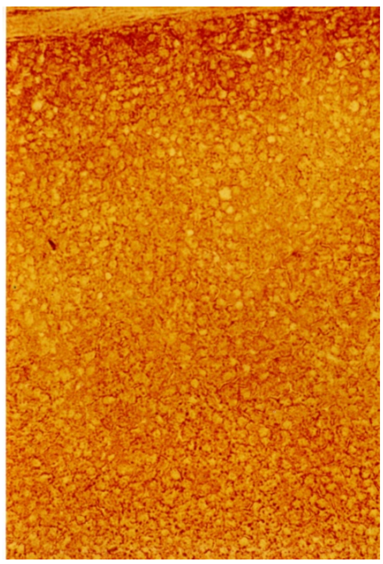

Figure 7. *Figure 8.* *Figure 9.*

Third group of animals

The third group of animals were given vitamin B6 prior the training

Figure 7 – glutamate dehydrogenase is mostly negative in the zona glomerulosa, and positive in the zona fasciculata, 120x.
Figure 8 – weakly positive LDH reaction in the zona glomerulosa, strongly positive in the outer part of the fasciculate and moderately positive in the remaining areas, 120x
Figure 9 – AcPh shows a weakly positive reaction in the zona glomerulosa and fasciculata, 120x

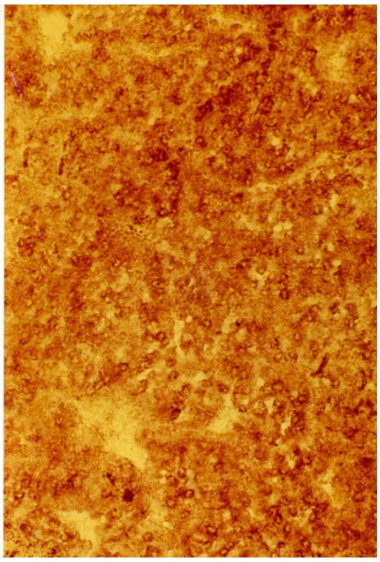

Figure 10.

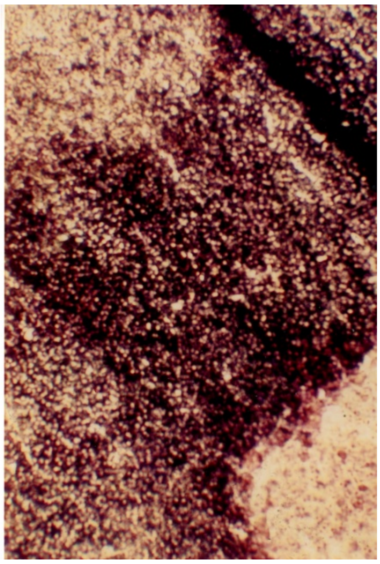

Figure 11.

Figure 12.

Fourth group of animals

The fourth group of animals was given vitamin B 12 prior the training.

Figure 10 – positive reaction to glutamate dehydrogenase is present in both zonas of the cortex, especially in the outer third of the zona fasciculata, 120x
Figure 11 – a very discretely present reaction to LDH in the zona glomerulosa and strongly positive in the zona fasciculata, especially in its inner part, 120x.
Figure 12 – a weakly positive reaction to AcPh in the zonas glomerulosa and reticularis, and negative in the zona fasciculata, 120x

The second group of animals that were given vitamin B1 prior the training, showed a weakly positive reaction to glutamate dehydrogenase in the zona glomerulosa, but significantly positive in the outer parts of the zona fasciculata (Figure 4). In the same group of animals LDH reaction was more positive in the zona fasciculata (Figure 5), while AcPh was only weakly positive in the zona glomerulosa (Figure 6).

In the suprarenals of the animals that were given vitamin B6 prior the swimming training, only a positive reaction of the glutamate dehydrogenase was present in the zona glomerulosa (Figure 7), very positive LDH reaction in the outer part of the zona fasciculata and positive in the remaining part (Figure 8), while the reaction of AcPh was weakly positive.

In the last group of trained animals treated with vitamin B12 there was a positive reaction to glutamate dehydrogenase, especially in the outer third of the zona fasciculata (Figure 10). LDH activity was less positive in the zona glomerulosa, but it was very positive in the zona fasciculata (Figure 11). Acid phosphatase was negative in the zona fasciculata, and weakly positive in the remaining zones (Figure 12).

Discussion

Since the first papers on the phenomenon of stress by Hans Seyle, the impact of stressogenic factors on metabolism, function and diseases of the body has increasingly occupied the researchers, namely because of the pressure of contemporary civilization, including considerable harmful environmental impact on human health, but at the same time numerous studies have been aimed at helping the body to adapt to these effects (1). Toweissy et al. consider stress as one of the basic factors in the etiology of a number of diseases (16). Apart from this, people themselves have tried to find ways to relieve stress and to reduce its harmful effects by knowing the mechanisms of stress, thus being actively involved in enzyme system function, especially in the adrenal hormone synthesis. It has been noted that in highly trained athletes chronic moderate hypercorticism is a basic characteristic of adaptive changes to chronic overload (3,8). Undoubtedly, there are numerous signal mechanisms, such as growth hormone and interleukins, that are directly or indirectly involved in stimulation of hypothalamic-pituitary-adrenal axis, namely pituitary ACTH, with corticotropin-releasing factor, thus increasing levels of the stress hormone, namely adrenal production of cortisol (3,6) since the levels of cortisol in circulation correspond to the levels of stress (17). In such a way interaction between nervous and endocrine system occurs (6). At the same time, stress causes a dysfunction in the normal circadian rhythm of adrenal hormone cortisol secretion (6). Prolonged stress may increase adjustment abilities and in a way achieve some kind of balance in the organism, but only to a certain point when these mechanisms begin to weaken. However, the vitamins B1, B6 and B12 may compensate that weakness (6).

The hypothalamic-pituitary-adrenal axis HPA is closely related to endocrine units that make up the peripheral limbs of the stress system, although there are individual mechanisms of adaptation to stress (6). The hypothalamic-pituitary-adrenal axis has two neuroendocrine pathways that stimulate adrenal glands in response to stress. In this study we are not interested in its peripheral sympatho-adrenal medullary system pathway (18), but in the one related to cortex, that is glicocorticoids.

It is well known that cortisol synthesis and secretion depend on different vitamins intake. Vitamin C is necessary for its biosynthesis. It has been shown that vitamin C decreases in adrenal cortex at higher cortisol levels (19). Our experimental research on Wistar rats, when they were exposed to cold temperatures, showed no concentration change of the ascorbic acid in the serum, liver and adrenals, while higher temperature was more stressogenic and caused decrease in ascorbic acid concentration in the adrenals, significant increase in serum and unchanged values in the liver (6,20,21), because vitamin C plays an integral role in maintaining cortisol levels. Maintaining ideal cortisol, also known as 'the stress hormone' levels is critical for human health. For steroid biosynthesis, derivatives B3 (niacin) are also necessary, as well as B5 (pantothenic acid) and B9 (folic acid) that play an important role in maintaining steroid secretion from adrenal cortex. Effects of pantothemic acid deficiency are specifically related to decreased adrenal function both in humans and animals (22-25). Adreno-cortical insufficiency has also been noted due to the lack of vitamin B7 (biotin) (4).

B-complex vitamins play a multiple role in the body. But, deficiency of one of them occurs with deficiency of other B vitamins. Most of them are not stored in the body and must be supplied on a daily basis. They are also needed for carbohydrate metabolism. Deficiencies in any of B vitamins can occur in conditions of stress as well.

It has experimentally been shown that application of vitamins B1, B6 and B12 has protective role in stressful events and they exhibit antistress activity and antioxidant properties at the same time (6,26-32). Intravenous administration of ascorbic acid in combination with vitamins B1 and B6 improves glicocorticoid function of the adrenal gland and stimulates normalization of the rhythmic activity of the adrenal glands (32).

LDH has a role in glycolysis conversion from anaerobic to aerobic process. Levy showed significantly present activity of LDH in the adrenal cortex, especially in the outer part of the zona fasciculata and zona glomerulosa, which is consistent with our experimental results. The group of animals that were under physical exertion and received vitamin B12 showed significantly stronger activity of this enzyme in the zona fasciculate and zona reticularis.

Glutamate dehydrogenase activity is weakly expressed in the zona glomerulosa, and is positive only in the remaining parts of the adrenals. After systematic physical exertion, especially with addition of vitamins B1, B6 and B12, glutamate dehydrogenase activity is increased in all the zones of the cortex, especially in the zona fasciculata, indirectly indicating greater possibility of glicocorticoid synthesis along with improved process of steroidogenesis.

Acid phosphatase belongs to the group of nonspecific phosphatases. Significant differences of AcPh are present in the adrenals, depending on the type of the animals. In rats, the whole adrenal cortex is poor in AcPh. Slightly increased activity of this enzyme is noted in the adrenal cortex in animals that were exposed to physical exertion, but also received B6 and B12.

Positive results of experimental and clinical studies serve to support the function of the adrenal glands (6) and may be realized by addition of ascorbic acid and vitamins B1 and B6.

Conclusion

Adaptation of the adrenal cortex was shown in rats under the swimming training with addition of vitamins B1, B6 and B12 by significant increase in the activities of enzymes LDH, AcPh, and GD that participate in adrenal cortical hormone synthesis, which is a confirmation of positive response to stress.

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ISPITIVANJE UTICAJA VITAMINA B1, B6 i B12 NA ADAPTACIJU KORE NADBUBREGA KROZ PRAĆENJE NEKIH ENZIMSKIH SISTEMA KOD PACOVA TRENIRANIH PLIVANJEM

Dragana Veličković, Milena Veljković

Centralno mesto u reakciji na nadražajne uticaje spoljašnje i unutrašnje sredine imaju hormoni nadbubrežne žlezde. Ispitivali smo aktivnost enzima (LDH-lactat dehidrogenaze, GD-glutamat dehidrogenaze i KF-kisele fosfataze) u kori nadbubrežne žlezde pod uticajem vitamina B grupe i treninga plivanja.

U ispitivanim slučajevima zapaženo je znatno povećanje aktivnosti enzima i to u zoni fascikulata i retikularisu nadbubrežne žlezde. Fizičko opterećenje, kao jedna vrsta stresa, izaziva usavršavanje procesa steroidogeneze. Korišćeni vitamini učestvuju kao kofermenti u stvaranju i delovanju mnogih enzima.

Poboljšanjem enzimskog sistema u nadbubrežnim žlezdama životinja treniranih plivanjem, uz dodavanje vitamina B1, B6 i B12, dolazi do brže i dugotrajne produkcije hormona neophodnih u opštem adaptacionom sindromu. *Acta Medica Medianae 2014;53(2):33-39.*

Ključne reči: *kora nadbubrežne žlezde, enzimi LDH, glutamat dehidrogenaza i KF, vitamini B1, B6 i B12*

