It will be clear from Table 1 that hemoperfusion does not change the serum levels of C-reactive protein (CRP) or of pregnancy zone protein (PZP) in the blood serum, and these proteins, moreover, likewise were not found in eluates from the charcoal. Nevertheless, the CRP concentration in the blood serum of psoriasis patients fall significantly later, but this evidently only reflects the development of a clinical remission.

## LITERATURE CITED


1. Yu. M. Lopukhin and M. N. Molodenkov, Hemoperfusion [in Russian], Moscow (1978).
2. Yu. M. Lopukhin, M. N. Molodenkov, N. G. Evseev, et al., Vest. Dermatol., No. 1, 8 (1980).
3. D. D. Petrunin, I. M. Gryaznova, Yu. M. Petrunina, et al., Byull. Éksp. Biol. Med., No. 5, 600 (1978).
4. N. I. Khramkova and G. I. Abelev, Byull. Éksp. Biol. Med., No. 12, 107 (1961).
5. G. Mancini, A. Carbonara, and J. Neremans, Immunochemistry, 2, 235 (1965).


# EFFECT OF PYRIDOXINE, RIBOFLAVINE, AND GLUTAMIC ACID ON RAT LIVER AND SERUM LYSOSOMAL HYDROLASE ACTIVITY DURING TRAUMATIC STRESS


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Stress causes considerable disturbances of cell metabolism [1, 3, 4]. In particular, glutamic acid metabolism is suddenly interrupted on the path of formation of inhibitory intermediates and of succinate, accompanied by a marked deficiency of pyridoxine and riboflavine [2, 14, 15]. Disturbances of relations between excitation and inhibition arising under these circumstances in the CNS lead to qualitative changes in neuroendocrine regulation of metabolism [8]. In turn, changes in the hormonal status of the body affect different aspects of its physiological activity, including the functional state of the lysosomal apparatus of cells in various organs and tissues [7, 9]. This influence is manifested as destabilization of the lysosomal membranes and the outflow of large quantities of hydrolases into the cytoplasm, followed by their appearance in the systemic circulation.

The object of this investigation was to study the effect of pyridoxine, riboflavine, and glutamic acid on the functional state of the lysosomal apparatus of the liver cells and hydrolase activity in the blood in traumatic stress evoked by crushing of the soft tisses of the hind limbs in rats.

## EXPERIMENTAL METHOD

Experiments were carried out on 128 male Wistar rats weighing 250-280 g. The rats, divided into eight series with nine animals in each series, were given glutamic acid (0.25% of the diet) and a fourfold excess of pyridoxine and riboflavine (0.008 mg) *per os* through a special tube daily for 14 days [7]. On the 15th day, after starvation for 12 h, the soft tissues of the rats' thighs were crushed. The control consisted of 56 animals, divided into eight series with seven rats in each series. Trauma was applied in both experimental and control rats, by crushing the soft tissues of the hind limbs with special forceps, designed to a model suggested in the Department of Pathological Physiology, S. M. Kirov Military Medical Academy [4]. To evaluate the effect of a combination of pyridoxine, riboflavine, and glutamic acid on the lysosomal system of the rat liver cells more completely, a modified model of long-term crushing of the soft tissues was adopted: the forceps were not removed until the end of the experiment, so that the main mass of crushed thigh muscles was isolated from


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TABLE 1. Total (A) and Nonsedimented (B) Activity of Rat Liver Microsomal Enzymes after Crushing Soft Tissues and Administration of Excessive Quantities of Pyridoxine, Riboflavine, and Glutamic Acid (in μmoles/min/g wet weight of tissue) (M ± m)

| Experimental conditions | Time of crushing, h | Cathepsin C | | | | Cathepsin $B_1$ | | | | Aryl sulfatases A and B | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | | B | | A | | B | | A | B | | |
| | | control | experiment | control | experiment | control | experiment | control | experiment | control | experiment | control | experiment |
| Intact animals | | 4,78±0,27 | 7,80±0,56*** | 7,4±0,5 | 7,6±0,3 | 3,0±0,35 | 2,43±0,34* | 20,1±0,9 | 27,3±1,2** | 1,46±0,05 | 1,99±0,10*** | 1,8±0,1 | 1,70±0,09 |
| | | 5,51±0,65 | 6,09±0,66 | 6,2±0,3 | 8,6±0,4* | 10,43±0,22 | 1,67±0,28*** | 7,6±0,5 | 45,6±2,1*** | 2,89±0,06 | 1,58±0,05*** | 5,2±0,3 | 2,00±0,11*** |
| Short-term fixation | 1,2 | 6,64±0,29 | 6,98±0,31 | 4,6±0,2 | 6,9±0,3* | 8,76±0,29 | 1,80±0,13*** | 11,0±0,7 | 39,2±1,8*** | 2,73±0,07 | 1,85±0,03 | 5,3±0,3 | 2,60±0,11*** |
| | 1 | 16,04±2,62 | 6,75±0,54** | 19,0±1,2 | 7,7±0,4*** | 8,84±0,15 | 2,61±0,26*** | 7,3±0,2 | 13,5±1,4*** | 2,79±0,10 | 2,14±0,06*** | 5,1±0,2 | 1,84±0,10*** |
| | 2 | 11,42±1,45 | 6,55±0,43** | 22,0±1,1 | 14,6±1,1*** | 8,54±0,22 | 2,42±0,38*** | 7,7±0,2 | 26,2±1,1*** | 2,68±0,10 | 2,28±0,05** | 7,3±0,3 | 3,10±0,13*** |
| | 3 | 10,81±1,02 | 5,24±0,49*** | 42,9±2,3 | 8,0±0,5*** | 8,61±0,07 | 2,08±0,35*** | 10,5±0,4 | 36,2±1,5*** | 2,67±0,09 | 1,95±0,03*** | 8,5±0,5 | 1,80±0,09*** |
| | 4 | 13,08±0,73 | 7,29±0,18*** | 24,8±1,3 | 9,4±0,7*** | 8,31±0,29 | 2,83±0,29*** | 9,3±0,3 | 26,9±0,9*** | 2,66±0,04 | 2,01±0,06*** | 9,1±0,5 | 1,99±0,10*** |
| | 5 | 10,73±1,38 | 9,32±0,60 | 35,9±1,6 | 6,9±0,2*** | 8,98±0,07 | 4,25±0,41*** | 8,6±0,2 | 16,6±1,0*** | 2,76±0,05 | 1,85±0,03*** | 9,8±0,5 | 2,44±0,12*** |

| β-glucuronidase | | | | p-Acetyl-β-D-galactosaminidase | | | |
|---|---|---|---|---|---|---|---|
| A | | B | | A | | B | |
| control | experiment | control | experiment | control | experiment | control | experiment |
| 1,04±0,03 | 1,66±0,09*** | 4,12±0,19 | 4,34±0,18 | 18,29±0,90 | 26,51±1,34** | 2,78±0,13 | 1,91±0,07* |
| 1,12±0,04 | 1,19±0,04 | 4,07±0,21 | 6,91±0,26* | 14,57±0,46 | 24,31±0,78** | 1,69±0,11 | 2,54±0,12* |
| 1,02±0,03 | 1,38±0,02** | 4,68±0,20 | 5,59±0,28* | 9,44±1,08 | 23,09±0,59** | 2,27±0,11 | 2,67±0,14* |
| 1,01±0,07 | 1,65±0,04*** | 5,04±0,23 | 4,68±0,19 | 11,20±1,38 | 25,83±1,34** | 2,23±0,15 | 2,21±0,09 |
| 1,30±0,06 | 1,49±0,02** | 3,32±0,17 | 5,64±0,21** | 10,80±0,77 | 27,31±1,90** | 2,98±0,12 | 2,01±0,08* |
| 1,06±0,05 | 1,02±0,03 | 5,01±0,27 | 7,83±0,28* | 12,00±0,61 | 12,97±1,01*** | 2,10±0,09 | 2,29±0,09 |
| 1,18±0,05 | 1,25±0,04 | 5,59±0,23 | 7,05±0,31* | 13,12±0,46 | 25,37±0,78*** | 3,23±0,14 | 2,31±0,19* |
| 1,37±0,06 | 1,27±0,04 | 4,98±0,25 | 6,60±0,27* | 10,80±0,31 | 29,94±2,13** | 3,15±0,08 | 2,41±0,15* |

Legend. Here and in Table 2 mean values from 7-9 experiments are given. *P < 0.05; **P < 0.01; ***P < 0.001.

TABLE 2. Activity of Lysosomal Enzymes in Rat Blood Serum (in μmoles/min/ml) after Crushing of Soft Tissues Accompanied by Administration of Excessive Doses of Pyridoxine, Riboflavine, and Glutamic Acid (M ± m)

| Experimental conditions | Time of crushing, h | Cathepsin C | | Cathepsin $B_1$ | | Aryl sulfatases A and B | | α-Glucuronidase | | β-N-acetylgalactosaminidase | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | control | experiment | control | experiment | control | experiment | control | experiment | control | experiment |
| Intact animals | — | 17,73±1,98 | 16,94±1,10 | 12,82±1,28 | 11,27±1,34* | 0,567±0,011 | 0,307±0,012* | 0,272±0,013 | 0,477±0,013*** | 35,99±2,79 | 24,36±0,77*** |
| Short-time fixation | — | 22,88±4,17 | 21,33±2,74 | 8,12±1,57 | 7,55±0,73 | 0,623±0,018 | 0,440±0,012*** | 0,243±0,016 | 0,303±0,009*** | 33,52±4,44 | 32,99±3,73** |
| | 1,2 | 24,23±3,08 | 19,90±1,14* | 11,33±1,75 | 4,06±1,60*** | 0,609±0,010 | 0,450±0,013*** | 0,233±0,013 | 0,330±0,014*** | 46,16±2,19 | 37,13±1,55** |
| | 1 | 33,98±3,73 | 23,47±1,83* | 13,83±1,98 | 9,14±1,53* | 0,620±0,009 | 0,440±0,011** | 0,333±0,011 | 0,262±0,012*** | 49,48±3,69 | 33,28±5,13* |
| | 2 | 37,23±5,27 | 21,09±5,03* | 13,06±1,35 | 8,46±1,60* | 0,625±0,013 | 0,440±0,012*** | 0,227±0,011 | 0,203±0,010 | 45,92±3,40 | 38,21±3,57 |
| | 3 | 42,38±5,27 | 21,80±5,98* | 13,00±1,23 | 8,61±0,80** | 0,574±0,013 | 0,423±0,006*** | 0,170±0,007 | 0,191±0,009* | 45,68±1,69 | 41,43±2,87 |
| | 4 | 45,52±5,27 | 26,71±5,48* | 10,98±1,76 | 7,48±1,38* | 0,555±0,009 | 0,339±0,013*** | 0,160±0,010 | 0,211±0,006*** | 46,44±1,48 | 30,60±3,59** |
| | 5 | 33,19±4,17 | 30,53±5,40 | 13,83±0,82 | 9,67±0,22*** | 0,586±0,014 | 0,479±0,011*** | 0,270±0,013 | 0,202±0,005*** | 30,73±2,89 | 34,41±4,97* |

the rest of the body. The forceps were applied for 2-3 min and for 30 min, after which the duration of tissue crushing was increased by 1 h in each successive series. The whole period of trauma was 5 h in duration.

At the end of the period of tissue crushing the rats were decapitated, blood was collected, and the liver was immediately removed, thoroughly washed in cold physiological saline, weighed, and homogenized in a glass Potter–Elvehjem homogenizer with Teflon pestle in 0.25 M sucrose solution with 0.001 M EDTA [5]. All operations were carried out at 0-2°C. Serum was obtained after centrifugation of the blood for 30 min at 3000 rpm.

To determine total enzyme activity the liver homogenate was incubated for 3 min at 37°C with Triton X-100 in a final concentration of 0.1%. Tests of nonsedimented enzyme activity were carried out in the supernatant obtained after centrifugation of the liver homogenate at 105,000g for 30 min.

Activity of five lysosomal hydrolases was determined in the liver homogenate, supernatant obtained from it, and the blood serum of the rats: cathepsin C, cathepsin $B_1$, aryl sulfatases A and B, β-glucuronidase, and n-acetyl-β-D-galactosaminidase. Activity of cathepsins C and $B_1$ was determined spectrofluorometrically, using benzoyl-arginine-β-naphthylamide and L-glycine-phenylalanine-β-naphthylamide (from Sigma, USA) as corresponding substrates [13]. Activity of n-acetyl-β-D-galactosaminidase was determined spectrofluorometrically, using 4-methylumbelliferyl-β-D-galactosaminide as substrate [12]. Activity of aryl sulfatases A and B and of β-glucuronidase was determined by spectrophotometric micromethods using p-nitrocatechol sulfate (from Sigma, USA) and p-nitrophenyl-β-D-glucuronide (from Sigma, West Germany) as substrate respectively [6, 10].

## EXPERIMENTAL RESULTS

The results showed that administration of pyridoxine, riboflavine, and glutamic acid for 14 days to the rats caused an increase in the total activity of all enzymes by 1.3-1.6 times except cathepsin $B_1$, whose activity fell (Table 1). Values of nonsedimented activity of cathepsin C, aryl sulfatases A and B, and β-glucuronidase were virtually indistinguishable from the control values, evidence of the high stability of the lysosomal membranes (Table 1). This conclusion also was confirmed by the level of lysosomal hydrolase activity in the blood serum, which was close to the control for cathepsin C and $B_1$, and actually lowered a little in the case of aryl sulfatases A and B and p-acetyl-β-D-galactosaminidase by 46 and 52% respectively (Table 2). Meanwhile, however, β-glucuronidase activity was increased by 75% as a result of administration of the vitamins.

The predominant response of most of the lysosomal hydrolases to administration of vitamins $B_2$ and $B_6$ and of glutamic acid was thus an increase in their total activity in the liver, which could reflect a more rapid synthesis of these enzymes *de novo*. The more than threefold increase in nonsedimented cathepsin $B_1$ activity in the experimental animals suggests the possibility of a nonspecific action of cytoplasmic proteinases, induced by the increased pyridoxine concentration [11]. Evidence in support of this view is given by the fairly high (compared with the control) level of nonsedimented cathepsin $B_1$ activity at all stages of trauma.

The study of the time course of changes in lysosomal hydrolase activity during severe mechanical trauma, both in intact animals and in rats receiving the combination of vitamins, preventing an increase in the severity of the state of stress, reveals changes of a similar character in their activity both in the liver and in the blood serum. However, after administration of these substances a significant decrease was observed in nonsedimented activity of cathepsin C and aryl sulfatases A and B, which as early as 5 h after the beginning of trauma amounted to only 19 and 28% respectively of their level of activity in the control animals. Meanwhile, no such effect was found for β-glucuronidase and for n-acetyl-β-D-galactosaminidase.

The character of the change in activity of the enzymes studied in the blood serum was even more demonstrative. In animals receiving vitamins $B_2$ and $B_6$ and glutamic acid beforehand, activity of cathepsins C and $B_1$, was considerably reduced, whereas activity of β-glucuronidase differed only very slightly at all stages of trauma from the control values at the corresponding times. It should be noted, however, that with an increase in the period of crushing of the soft tissues the difference in enzyme activity between the control and experimental animals gradually disappeared. For instance, whereas by the second hour of trauma it was between 18 and 45% for all enzymes, by the 5th hour this difference did not exceed 8-30%.

The results suggest that during severe mechanical trauma due to crushing of the soft tissues pyridoxine, riboflavine, and glutamic acid can exert a marked protective action on the lysosomal apparatus of the liver cells, which is probably based on increased stability of the lysosomal membranes. With an increase in duration of the period of trauma the effectiveness of the substances used declines. This suggests that the mechanism of the effect of the vitamins on maintenance of the structural and functional integrity of the lysosomal apparatus of the liver cells is indirect, through the action of these substances on separate metabolic pathways in the nerve cells and limitation of the irradiation of excitation from the CNS, especially through the participation of their intermediates (GABA and GHBA) in the strengthening of inhibition in the brain. This "equalization" of the levels of enzyme activity observed in the control and experimental animals with lengthening of the period of trauma may correspondingly reflect the active utilization of the administered vitamins and, ultimately, their deficiency.

## LITERATURE CITED


1. A. P. Dovganskii, "Data on the pathogenesis of burns (experimental study)," Author's Abstract of Doctoral Dissertation, Kishinev (1971).
2. V. V. Efremov, in: Recent Data on the Therapeutic Use of Vitamins [in Russian], Moscow (160), pp. 159-169.
3. A. A. Zor'kin and V. I. Nigulyanu, in: The Pituitary–Adrenal Systems and Metabolism in Shock [in Russian], Kishinev (1977), pp. 15-69.
4. V. K. Kulagin, in: The Pathological Physiology of Trauma and Shock [in Russian], Leningrad (1978), pp. 161-178.
5. A. A. Pokrovskii and V. A. Tutel'yan, Biokhimiya, 33, 809 (1968).
6. A. A. Pokrovskii and A. I. Archakov, in: Modern Methods in Biochemistry [in Russian], Moscow (1968), p. 5-59.
7. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976).
8. A. A. Pokrovskii and K. A. Korovaikov, Vopr. Pitaniya, No. 5, 67 (1977).
9. M. I. Remizova and N. I. Kochetygov, Patol. Fiziol., No. 3, 63 (1976).
10. V. A. Tutel'yan, "Enzyme characteristics of lysosomes and changes in them under the influence of alimentary and toxic factors," Author's Abstract of Doctoral Dissertation, Moscow (1977).
11. N. Katanuma, Enzym. 1973, Berlin (1974), pp. 313-324.
12. S. Okada and Y. S. O'Brien, Science, 165, 698 (1969).
13. T. G. Peters, M. Müller, and C. de Duve, J. Exp. Med., 136, 1117 (1972).
14. E. Roberts, Am. J. Clin. Nutr., 12, 291 (1963).