Antioxidant Enzyme Levels in Red Blood Cells of Chronic Alcoholic Men Patients During Oral Niacin Therapy

Irene Durko[1], Erzsebet Papp, M.D.[2], Ilona Sz. Varga[3], Ilona Balogh[1], Peter Szerdahelyi[4], Laszlo Maroti[2], Denes Budai[4], Laszlo Raska, M.D.[2], Janos Szilard, M.D., Ph.D.[1]

Oral niacin therapy (Vitamin B_3) has given promising results[2] in the treatment of so-called NAD deficiency diseases such as alcohol and opiate addiction.[3].

Niacin as a co-factor of ethanol metabolism participates in the improvement and maintenance of NAD/NADH+H concentration ratio of chronic alcoholic patients with energy poor state.

The value of this ratio is decisive not only in the two-step oxidation of ethanol by alcohol-dehydrogenase (ALD) and alde-hyde-dehydrogenase (ALDH) isoenzymes, but it also affects the catalase and the microsomal ethanol oxidating system (MEOS) through redox changes. The latter serves to maintain equilibrium by decreasing the "reducing equivalent" ($NADH+H^+$).

Its effect is not negligible either, since it affects the stability of Schiff-bases formed from the reaction of acetaldehyde and with different amino, hydroxyl, and SH content biologically active substances (e.g. proteins, neurotransmitters, etc.). Increase of $NADH+H^+$ concentration favours the formation of stabile Schiff-bases reducing the first step product acetaldehydeadduct.[11]

The formation of stabile Schiff-bases, on the other hand, promotes the elimination of toxic acetaldehyde present occasionally in critical concentration, and at the same time it also helps in the synthesis of several opoid compounds, e.g. tetrahydro-isoquinolines,[4] tetrahydro-beta-carbo-lines, etc.

The role of the latter is still debated and widely studied, in relation to alcohol consumption, alcohol addiction, and in the formation of brain- and neural- lesions. It is an accepted fact that the formation of stabile Schiff-bases is responsible for the disturbed monoamine-metabolism of alcoholics.

Increase of "reducing equivalent" changes the ratios of oxidized and reduced glutathiones and is important in the oxidation of ethanol in the liver. The resulting decrease in pH affects the lipid-peroxidation, and the activity of antioxidant en-zymes.

The aim of our study was to measure the levels of antioxidant enzymes in chronic alcoholic male patients in different periods of niacin therapy. We wanted to know how orally administered niacin in therapeutically active doses influenced intracellular enzymes functioning as a first line of defense in chronic alcoholic individuals.

In a self-controlled experiment with accurately selected patients under closed clinical conditions the degree of lipid-peroxidation (LPO) in red blood cells, and levels of superoxide-dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GP) enzymes were determined at therapeutically effective levels of B_3 (1,320 mg/day) and at "maintenance" dose (660 mg/day) which prevents relapse.

Our studies were complemented with the simultaneous measurements of Se, Fe^{++}, Cu^{++} and Zn^{++} -ion concentrations considering the metal-ion sensitivity of antioxidant enzymes.

1. Department of Neurology and Psychiatry, Albert Szent-Gyorgyi Medical University, 6. Semmelweis str. 6722 Szeged, Hungary.
2. Alcohol withdrawal and Work-therapy Institute. Nagyfa, Hungary.
3. Biological Izotop Laboratory Attila Jozsef University of Sciences, 52. Kozfcpfasor, 6726 Szeged.
4. Central Laboratory for Research. Albert Szent-Gyorgyi Medical University, 4. Somogyi str., 6721 Szeged.

Experiment and Methods

The study was carried out at the Alcohol-withdrawal and Work-therapy Institute (Nagyfa, Hungary) between 1987-1989, on 40 chronic alcoholic volunteer men (average age of 36.8 yrs.). The mean duration of their alcohol consumption was 13.5 years.

In selecting persons to be tested liver function tests below (gamma GT 28.0 ± 9.12 U/1, SGOT 7.41 ± 3.34 U/1, TG 1.38 ± 0.45 mmol mean values) are decisive. Persons with diabetes, TBC, significant myocardial lesion, and gastrointestinal or hematopoietic disease were excluded from the investigation.

Subjects of the investigation were of average or high intelligence according to Wechsler's performance test. Except for the particular personality aspects of alcoholism, no substantial deviations could be found with thorough psychiatrical and/or psychological tests (MMPI, Taylor's-, Bus-Durkee's tests, neurosis and mood scale) performed.

Each person was tested in four periods:

I. period: Right after admission to the Institute during abstinence.

II. period: After one month abstinence, without vitamins.

III. period: After one month oral administration of niacin (2x3 tablets/day; 1,200 mg + 120 mg (in Poly B) nicotinamide). Vitamins: Poly B, A, E, C.

IV. period: After three months oral administration of niacin (3x1 tablets/day; 600 mg + 60 mg (in Poly B) nicotinamide). Vitamins: Poly B, A, E, C.

The patients received the following types of vitamins:

Poly B: In the III. period 2x3 tablets/ day; IV. period 3x1 tablets/day. Each tablet contains thiamin chloride 5 mg, riboflavin 2 mg, nicotinamide 20 mg, pyridoxin chloride 20 mg.

Vitamin A: In the III. period 3x1 tablets/day. 1 tablet contains 300 IU retinolum EGIS-BIOGAL, or 2x1 capsule/week, one capsule contains 50,000 IU. In the IV. period 1 tablet/week (3,000 IU).

Vitamin E: In the HI. period 1 tablet/ day, one tablet coptains 100 mg tocopherol acetate. In the IV. period 1 tablet/week.

Vitamin C: In the III. period 1 tablet/ day, each tablet contains 100 mg ascorbic acid. In the IV. period 1 tablet/week.

Venous blood samples were taken between 8-9 in the morning, LPO[9], and enzyme activities of SOD,[6] CAT,[1] GP,[5] respectively, protein concentration (Low-ry) and Se Fe^{++}, Cu^{++}, Zn^{++} - ion concentrations with atomic absorption method were determined. Serum gamma GT, SGOT and TG values were always measured simultaneously.

Acetaldehyde and the occasional ethanol concentrations were also measured with gas-chromatography (Christensen et al, 1981) in the same blood sample, so as to check the state of abstinence. In spite of the strictly controlled conditions of the institute, secret alcohol consumption occurred occasionally in the IV. period, due to alcohol dependency — never though in the time the blood sample was taken.

Calorie uptake of our patients was approximately uniform during their treatment at the Institute.

Results (Figures and Tables p. 206-209)

Data demonstrating the liver conditions of our patients treated with orally administered niacin are summarized in Table 1.

Table 1 shows the fairly good effect of abstinence on the state of liver metabolism. Administration of 1,320 mg niacin daily proved to be the most favourable in the formation of gamma GT level, while it did not show the same favourable trend in case of SGOT. After three months of treatment, levels of both enzymes increased.

Triglyceride levels (TG) in each case were within the normal values, though niacin caused a slight increase. Changes in serum Fe^{++}, Cu^{++} and Zn^{++} ion concentrations at the times of checking are shown in Table 2.

Except for the initially high Fe^{++} ion concentration, the levels of these ions were within the normal values. Favourable impact of abstinence was expressed by the slight increase of Fe^{++} and Cu^{++} ions affected by niacin, while a decreasing trend of Zn^{++} ion-concentration (within the normal values) was observed. (We found significant differences in Fe^{++} ion between the I. - II. periods only.)

Blood acetaldehyde measurements with gas-chromatography gave the following results during the tests of abstinence.

Periods	Mean values
I.	1.26 ± 0.45 /µmol/l
II.	1.23 ± 0.43 /µmol/l
HI.	1.99 ± 0.67 /µmol/l
IV.	2.62 ± 0.70 /µmol/l

Since no ethanol could be measured, abstinence should be assumed. Literature data indicate 2.1 ±1.7 umol/1 as normal value. The effect of niacin is striking, though we do not know whether the slight increase in acetaldehyde level could be attributed to the inhibition of ethanol metabolism or of xanthine oxidase or to the increase of the amount of unstable Schiff-bases. The latter contradicts the fact that Vitamin C has a stabilizing effect on Schiff-bases.[11]

Results obtained on lipid peroxidation level are shown in Figure 1.

One-month-long oral administration of niacin in amounts of 1,320 mg/day had a favourable effect on lipid peroxidation. While the abstinence itself did not result in a significant recovery, niacin of maximum dose, combined with high dose vitamin therapy resulted in a significant decrease. This favourable tendency did not prevail in niacin at the lower level, 660 mg/day, even in case of parallel Vitamin therapy.

Level of glutathion-peroxidase enzyme obviously improved in abstinency (Figure 2). Antioxidant defense decreased upon niacin therapy, which, however, improved in the IV. period administered in lower concentrations. Our data confirm the earlier observation of Moore and Goldberg,[7] that serum transferases — gamma GT in our investigations — show linear correlation with the glutation-peroxidase values of liver (cf. Table 1).

Changes in catalyse enzymes in the periods I-IV are summed up in Figure 3.

Low concentrations in chronic abusers was found in 59% of patients in the I. period. The effect of abstinence could be observed since 57% of the persons tested had normal activity values in the II. period. Effect of niacin can be considered positive in the III. and IV. periods as well. We presume that the really abstinent state of our patients was confirmed by the catalyse enzyme activity values measured during the investigation.

Figure 4 demonstrates the distribution of superoxide dismutase (SOD) enzyme levels responsible for elimination of reactive superoxide radicals. No change could be observed upon the abstinence; on the other hand, niacin administered in maximum dose adversely influenced the antioxidant defense provided by SOD.

Discussion

From the results presented here, we consider the significant decrease of lipid-peroxidation important in the III. period. We have observed positive changes in lipid-peroxidation contrary to the marked decrease of enzyme defense by antioxidant enzymes. Literature data concerning the processes triggering lipid peroxidation suggest that the inhibition of iron-catalysed, so-called Haber-Weiss reaction by adenyl-nucleotides which form chelates with iron, might have a role in the patients tested in our investigation ($H_2O_2^{+}O_2^{-iron}$ $OH+OH^{-}+O_2$).[12] Thereby we presume, that the quantity of OH' radicals which trigger lipid-peroxidation was decreasing.

The role of A, E and C vitamins, as natural antioxidants might be considered important in this effect too, since they inhibit the intermediate-steps of lipid peroxidation through well-known mechanisms. It is supported by the fact that high lipid peroxidation values observed in the IV. period might be the result of lower niacin and vitamin doses, though theievel of SOD enzyme increased.

As it is known, peroxidation of unsaturated fatty acids induces structural and functional changes in cell membrane,[8] which naturally also involves neural cells.[10] Lipid peroxidation might induce inhibition of e.g. dopamine synthesis, as it was shown by experiments of Zaleska et al[13] on striatum synaptosomes. The good therapeutical results with niacin observed in "NAD deficiency diseases" can be attributed to high doses applied and to the simultaneous vitamin therapy of mega-doses where niacin administered in lower form improved the therapeutical effect.

We suppose that the change in membrane structure caused by decreased lipid peroxidation in chronic alcoholics might play an important role in the therapeutic effect of niacin.

In presenting our results obtained during

niacin therapy of chronic alcoholics, our aim was to demonstrate that it is reasonable to test the antioxidant enzyme levels of red blood cells of the patients periodically and that the application of niacin and vitamins in "adequate doses" is really important in therapeutics.

Since our tests, unfortunately did not focus on the measurement of therapeutical effects which we deeply regret — no data can be given as to the potential relationship of lipid peroxidation level to therapeutic effects in chronic alcoholics. Several experiments in animals have been recorded in the literature confirming the role of "iron catalyzed processes" and "redox active iron content" in the development of physical dependence on alcohol.

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Figure 1
Ratio of lipidperoxidation in red blood cells during I-IV periods

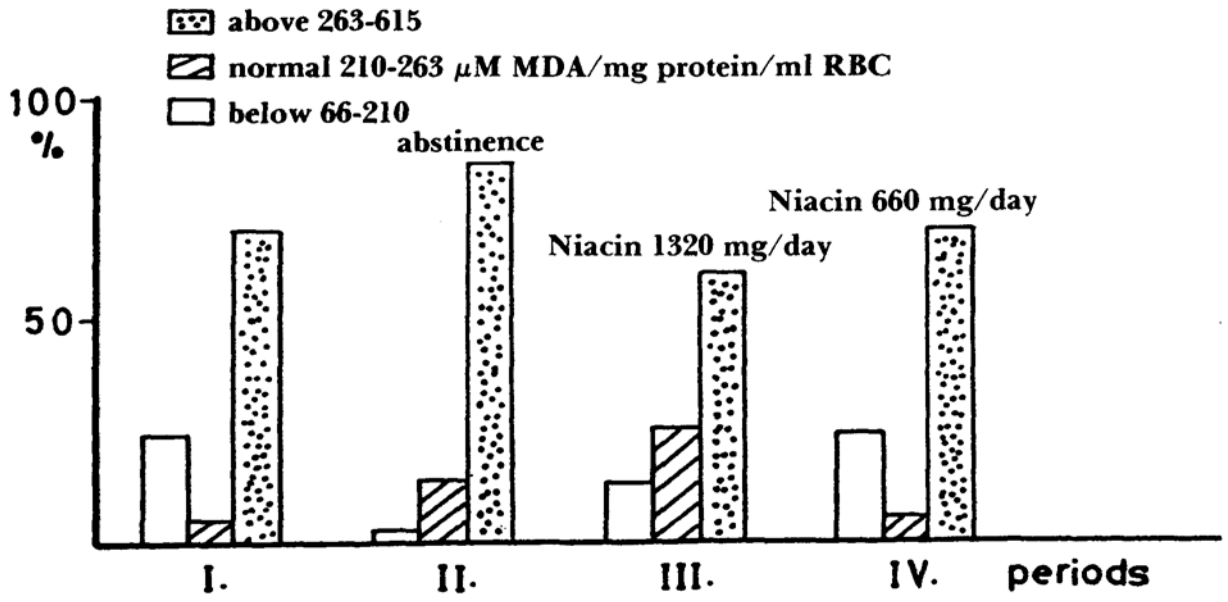


Figure 2
Ratio of glutathione-peroxidase enzyme levels in red blood cells during I-IV periods

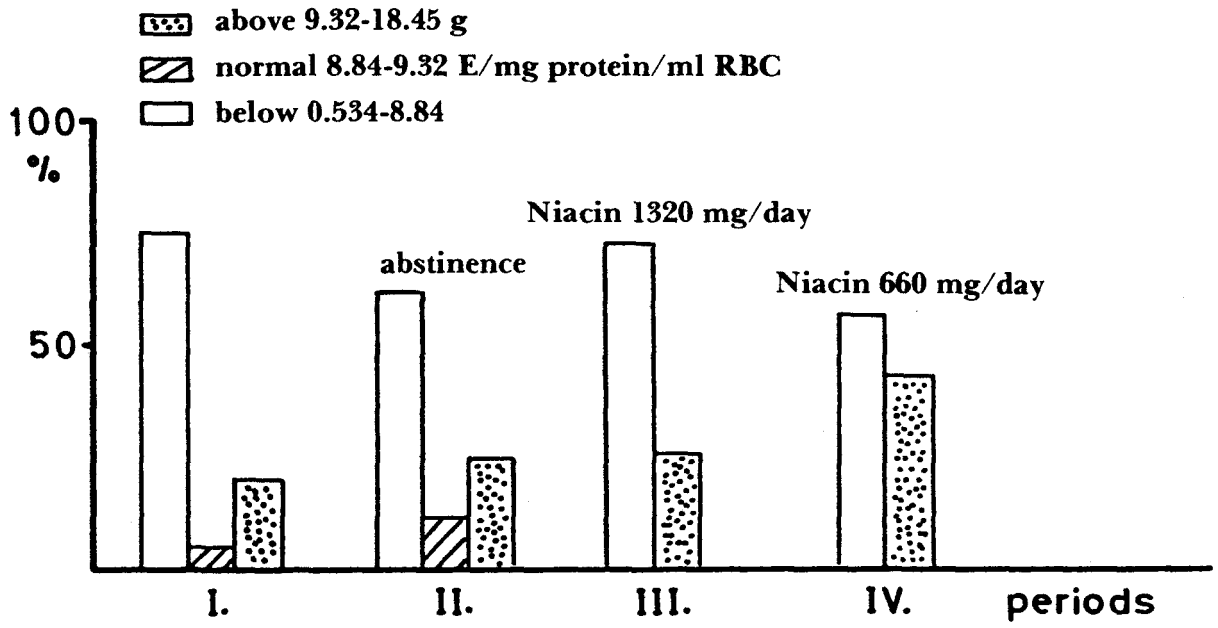


Figure 3
Ratio of catalase-enzyme levels in red blood cells during I-IV periods

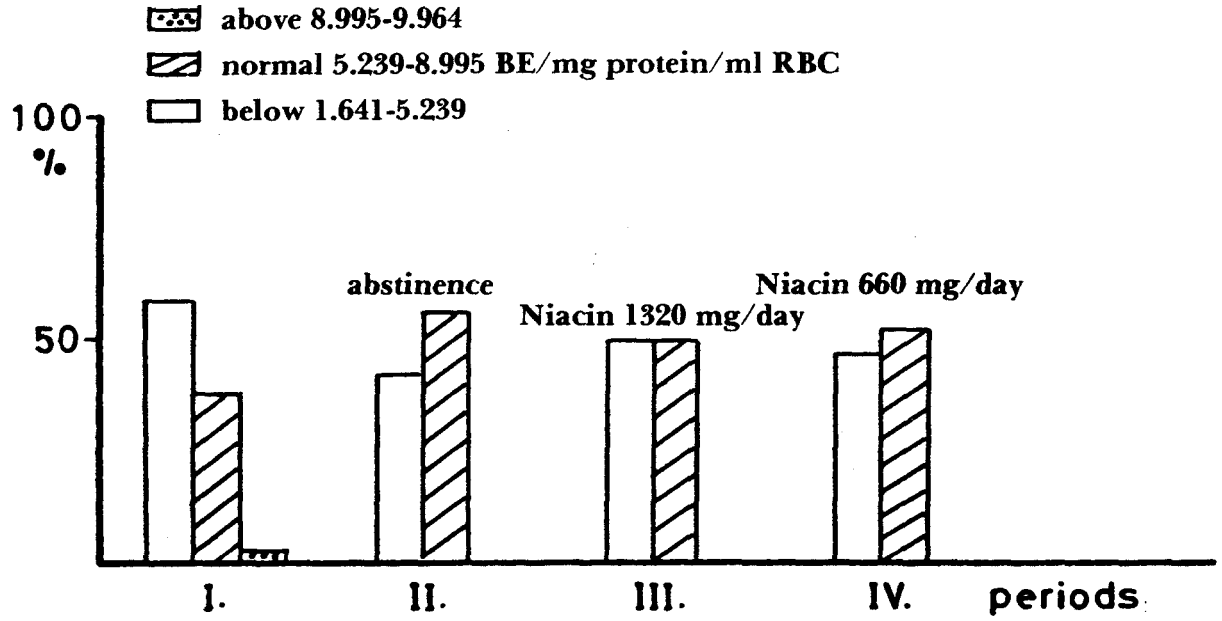


Figure 4
Ratio of superoxide-dismutase enzyme levels in red blood cells during I-IV periods

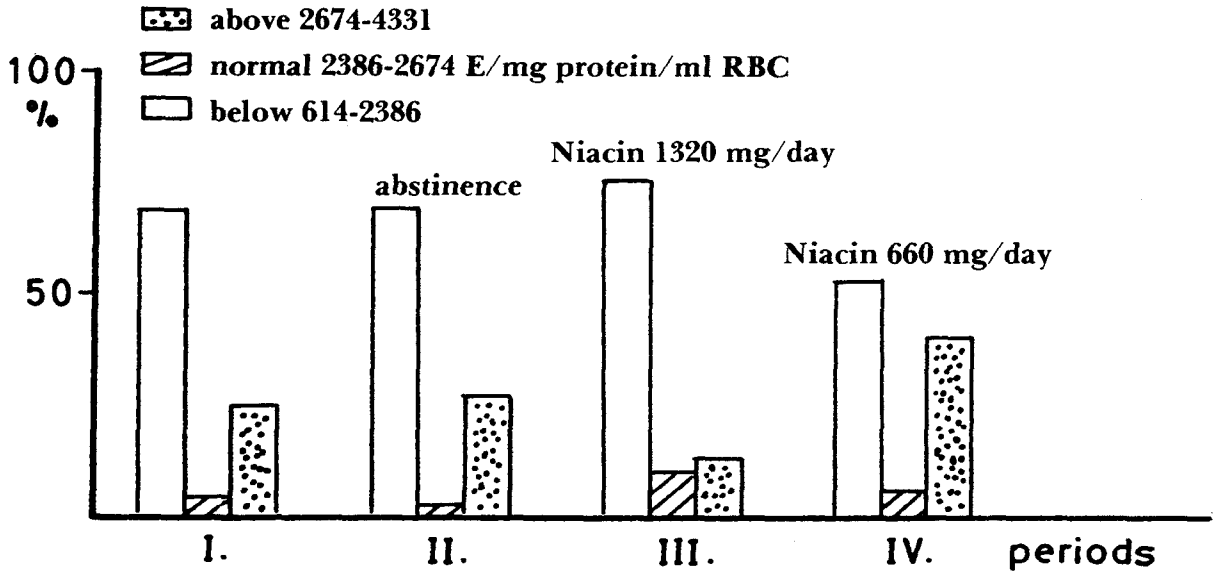


Table 1
Serum TG, SGOT and Gamma GT Levels During I-IV. Periods (N = 40)

Period	Trigliceride (TG) Normal; 0-1, 71 mmol/1	Glutamic-Oxaloacetic Transaminase (SGOT) Normal; 1-12 U/1	L-gamma-Glutamyl-Transferase (gamma GT) Normal; 6-28 U/l/men
I.	Mean; 1.38 ±0.45 Normal level; 90.62% above; 9.37%	Mean; 7.41 ± 3.34 Normal level; 90.32% above; 9.67%	Mean; 28.0 ±9.12 Normal level; 66.66% above; 33.33%
II.	Mean; 1.30 ±0.36 Normal level; 93.54% above; 6.45%	Mean; 4.8 ±2.1 Normal level; 93.54% above; 6.45%	Mean; 23.93 ± 7.99 Normal level; 70.0% above; 30.0%
III.	Mean; 1.45 ±0.43 Normal level; 90.62% above; 9.37%	Mean; 9.09 ± 3.98 Normal level; 70.96% above; 29.0%	Mean; 13.73 ± 5.63 Normal level; 86.20% above; 13.73%
IV.	Mean; 1.41 ±0.41 Normal level; 93.75% above; 6.25%	Mean; 11.8 ±4.68 Normal level; 80.0% above; 20.0%	Mean; 34.35 ±11.75 Normal level; 35.71% above; 64.28%

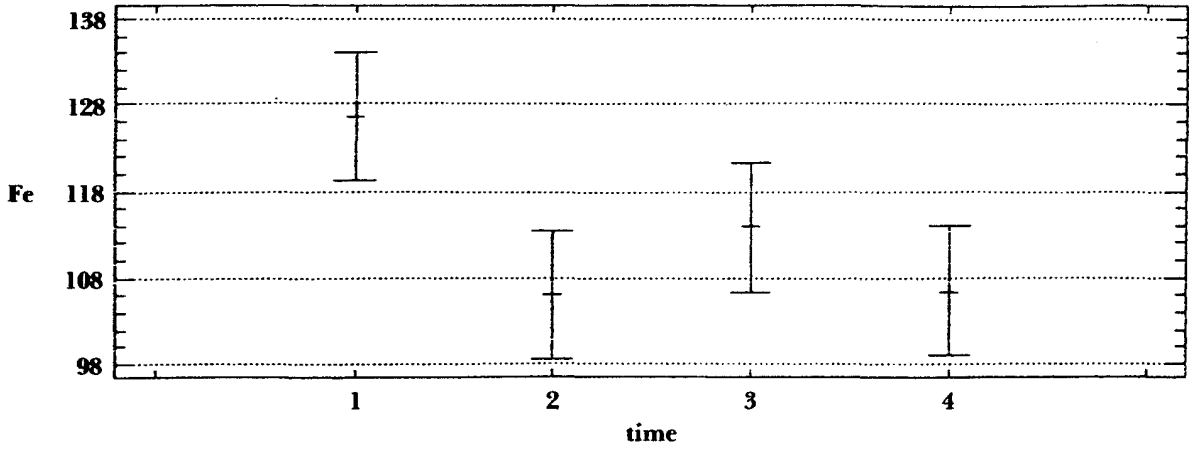
Methods: TG Boehringer UV-test/A. W. Wahlefeld in. H.U. Bergmeyer. Methoden der enzymatischen Analyse., 3 Aufl. Bd. II. Verlag Chemie Weinheim. 1974. s. 1878.

SGOT Boehringer-colorimetric method. Reitman, S. and S. Frankel (1957) Amer. J. Clin. Path. 28, 56.

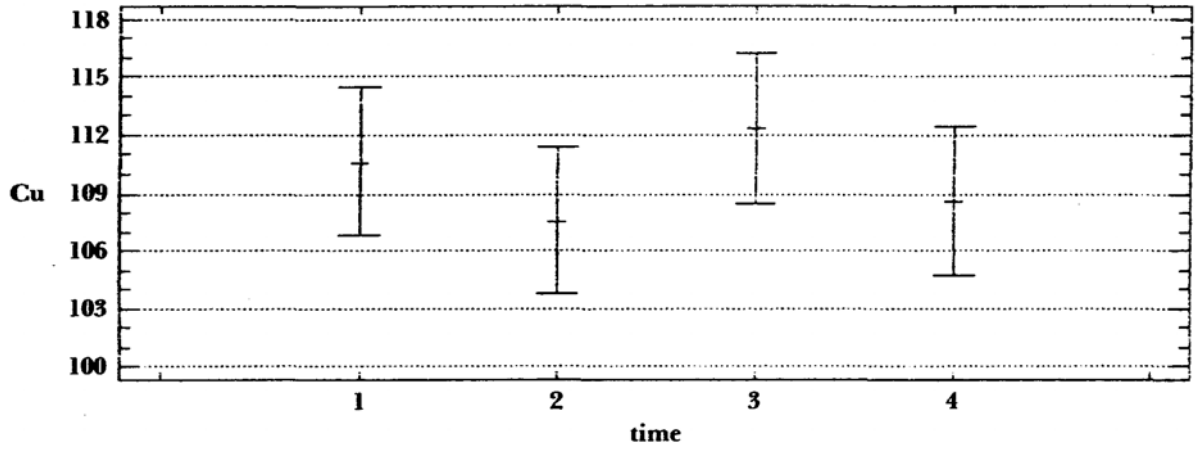
Gamma GT Boehringer monotest. Szasz, G., Persijn, J.P. et al. (1974) Z. Klin. Chem. Klin. Biochem. 12,228.

Table 2
Changes of Serum Fe^{++}, Cu^{++}, Zn^{++} Ions During I-IV. Periods

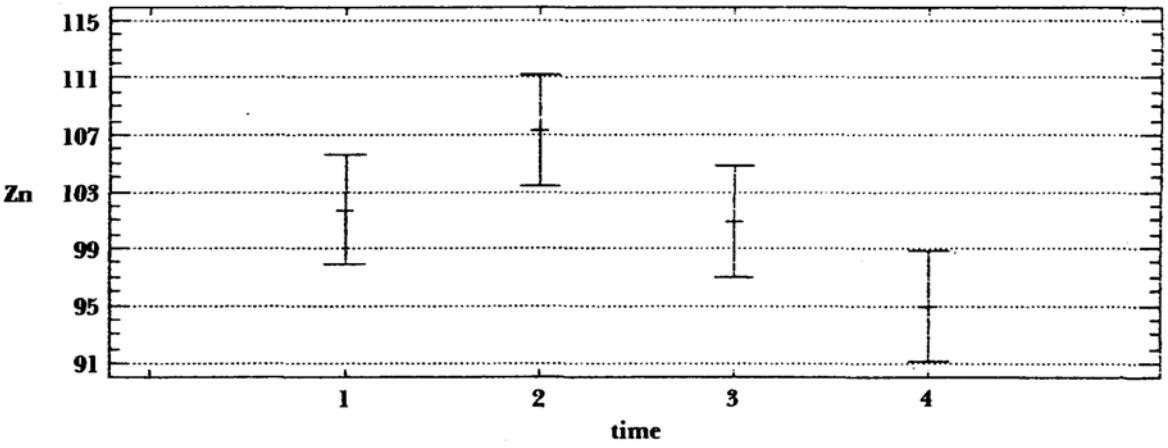
95 Percent LSD Intervals for Factor Means



95 Percent LSD Intervals for Factor Means



95 Percent LSD Intervals for Factor Means



Analytical method: Fernandez, F.J., Kann, H.L.: Clin. Chem. Newsletter. 3,24-27 (1971). Mathematical method: Analysis of variance.