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EFFECT OF CHEMICAL AGENTS ON SOME ENZYME ACTIVITIES AND ON THE STABILITY OF MEMBRANE STRUCTURES

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Summary

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The effects of two chemical agents − sodium nitrite and phenobarbital (PB) applied separately or in combination, on the activity of enzymatic components of monooxigenase system of hepatocytes, the stability of liver lysosomes and peroxisomes as well as on the total activity of marker enzymes for these structures were investigated in male rats.

Following the combined administration of both agents, the effects of sodium nitrite predominated. Nitrite eliminated at a considerable extent the membrane-stabilizing effect of the barbiturate, decreased its activating influence on aniline hydroxylase and potentiated PB-induced activation on systems catalyzing lipid peroxidation.

Key words: $NaNO_2$, phenobarbital, endoplasmic reticulum, enzyme activities, membrane stability

INTRODUCTION

There are numerous data indicating that after the purposive treatment with a given chemical agent, a combined rather than an individual effect is detected because of the unintentional pretreatment of humans or animals with various environmental contaminants. The existing reports show that the background is able to potentiate or antagonize the effect of a tested substance. In some cases, even an inversion of the effect is observed (Pavlova *et al.,* 1978; Popov *et al.,* 1990; Anjum & Shakoori, 1993; Dhawan & Goel, 1994; He & Wang, 1995; Popov *et al.,* 1996; Liu *et al.,* 1998; Popova *et al.,* 1999).

The endoplasmic reticulum (ER) is one of the main sites of cells where the metabolism of exogenic and endogenic low-molecular substances takes place. The monooxigenase system (MOS) is to a great extent responsible for this biotransformation (Conney, 1972; Mannering**,** 1972; Elves *et al.,* 1985; Hodgson *et al.,* 1995; Koleva *et al.,* 1999; Boll *et al.,* 2001). The activity of MOS as a whole or of its components may change the effect of chemical agents. Some MOS components participate in lipid peroxidation, a process that is activated under several conditions and that impairs the structure and function of membranes and of other cellular components (Banerje *et al.,* 1999; Popova & Popov**,** 2002).

The existence of numerous unresolved problems related to membrane stability and to altered function of ER motivated our interest to the effect of the combination sodium nitrite ($NaNO_2$) and pheno-

barbital (PB). These compounds are applied in several areas and are important because of their versatile effect on the organisms, so they have been and are still intensively studied (Popov *et al.,* 1979; Langlois & Calabrese, 1992; Bruning-Fann & Kaneene, 1993; Popov *et al.,* 1996; Meyer & Hoffmann, 1999; Mochizuki *et al,* 1999; Pavlov *et al.,* 1999; Popova, 2004).

Literature data (Conney, 1972; Popov *et al.*, 1979; Madhukar & Matsumura, 1981; Anjum & Shakoori, 1993; Meyer & Hoffmann, 1999) demonstrate convincingly that PB is a strong inductor of enzymes, metabolizing different chemical substances. It is also known that nitrites are transformed into nitrosamines by enzymes, located in the ER (Paul & Illing, 1981), and that these products exert a toxic (membrane-active, carcinogenic etc.) influence (Popov *et al.,* 1982; Popova *et al.,* 1982; Bradberry *et al.,* 1994; Popov *et al.,* 1996; Griffin, 1997; Popova *et al.,* 1999).

That is why, in order to elucidate some aspects of nitrate-nitrite effect, we performed investigations using PB as an agent, inducing an altered background via its effect upon the functional activity of the ER. The changes in some hepatocyte MOS components (aniline hydroxylase and lipid peroxidation), in liver lysosomes' and peroxisomes' stability and the total activity of marker enzymes for these structures were followed out.

MATERIALS AND METHODS

The studies were performed on 68 male Wistar rats weighing 170–200 g at euthanasia. During the experiment they were housed at 20–24 ^{o}C, had free access to drinking water and were fed with a commercial food for this species.

The rats were divided into 4 groups: group 1 – controls (untreated); group 2 – rats that received a food supplemented with 0.3% $NaNO_2$ for 7 days (group 2a) and 14 days (group 2b); group 3 – rats that received PB solution by stomach tube at 100 mg/kg using the following schedule: days 1, 2, 3, 5 and 7 (group 3a) and days 1, 2 and 3 and afterwards, every third day up to day 14 (group 3b). The rats from group 4 received food with $NaNO_2$ as in groups 2a and 2b and were treated with PB as in groups 3a and 3b (groups 4a and 4b respectively).

At the end of each experimental period, both control and experimental rats starved for 12 h but had a free access to drinking water. The euthanasia of all animals occurred via decapitation 48 hours after the last treatment of experimental rats with PB. The livers were obtained for analysis. They were immediately put into ice cold 0.25 M sucrose solution and homogenized in the same solution under cooling regimen. Liver homogenates were submitted to fractionation by differential centrifugation (Popov, 1976; Popov *et al*, 1976) and were used for analysis of ER enzymatic activities and the membrane stability of lysosomes and peroxisomes.

The activity of some MOS components of ER – aniline hydroxylase (AH) and lipid peroxidation (LP) was determined. The AH activity was assayed in post mitochondrial 20000 g supernatants by the method described by Popov (1976) and the enzymatic (NADPH-dependent) and non-enzymatic (ascorbate-dependent) systems, catalyzing LP – by a method described by Popov (1976) and Popov *et al.* (1976). The malonedialdehyde (MDA) concentration was calculated using the molar extinction coefficient 1.56×10^5 $cm^2.mmol^{-1}$ (Wills, 1969).

The stability of lysosomes was evaluated through the distribution of their marker enzyme acid phosphatase (AP) among the fractions obtained after the differential centrifugation of tissue homogenates. The non-sedimentable and "free" AP activities were determined. The criterion rate of AP release after preincubation of granule fractions in isotonic medium (0.25 M sucrose) at 37 ^{o}C and pH 5 (0.1 M acetate buffer) was also used. The AP activity (EC 3.1.3.2) was determined by the method of Gianetto & De Duve (1955). Peroxisome stability was assessed on the basis of catalase distribution among the homogenate fractions. The activity of peroxisomal marker catalase (EC 1.11.1. 6) was assayed according to Cohen *et al.* (1970).

The total activities of AP and catalase in homogenate fractions were determined in incubation media, containing Triton X-100 (0.1% and 0.4% respectively). The protein content of homogenate samples was estimated by the biuret method (Popov, 1972).

The statistical significance of changes in treated rats was determined by means of the Student t-test.

Table 1. Aniline hydroxylase activity in post-mitochondrial supernatants obtained from rat liver homogenates (mean ± SEM)

Groups	μg p-AMP/ mg protein/ 20 min
1. Controls (untreated) (n=9)	0.60±0.06
2a. $NaNO_2$ in food for 7 days (n=5)	0.64±0.06
2b. $NaNO_2$ in food for 14 days (n=4)	0.64±0.06
3a. Treated with PB for 9 days (n=4)	2.24±0.44**
3b. Treated with PB for 16 days (n=4)	1.52±0.12*
4a. $NaNO_2$ for 7 days + PB for 9 days (n=4)	1.52±0.12*
4b. $NaNO_2$ for 14 days + PB for 16 days (n=4)	1.40±0.16*

The treatment with phenobarbital (PB) orally at 100 mg/kg was as followed: by days 1, 2, 3, 5 and 7 (groups 3a and 4a) and by days 1, 2, 3, and afterwards every third day until the 14th (groups 3b and 4b). The euthanasia was done 48 h after the last treatment. "n" = number of the separate experiments; p-AMP = p-aminophenol; * $P < 0.01$, ** $P < 0.05$ vs controls.

RESULTS AND DISCUSSION

Table 1 shows the changes in the activity of AH after the treatment with agents studied. After 1-week and 2-week treatment with nitrite, the enzymatic activity was insignificantly higher. The PB however, increased AH activity several times (groups 3 and 4) . The differences vs the controls were statistically significant ($P<0.05$) when the drug was applied to animals receiving usual food (groups 3a and 3b). Another peculiarity was also present: after a more prolonged treatment (16 days) (group 3b), the activating effect of PB was less expressed than after a 7-day treatment (group 3a).

The tested substances exerted an influence on systems that catalyzed the LP too (Table 2). The effect of nitrite (groups 2a and 2b) was comparatively low – $NaNO_2$ only activated weakly the enzymatic system after the 7-day treatment but not after the 14-day one. In previous studies of ours, we established that both nitrites and nitrates were strong stimulators of LP. This effect was however manifested after a more prolonged treatment (20–60 days

Table 2. Activity of the enzymatic (1) and nonenzymatic (2) systems, catalyzing lipid peroxidation, determined in postmitochondrial supernatants obtained from rat liver homogenates (mean ± SEM)

Groups	nmol MDA/mg protein/30 min			
	7-day experiment		14-day experiment	
	1	2	1	2
1. Controls (untreated) (n=9)	39.7±3.8	41.7±5.8	28.2±3.8	34.6±10.3
2a. $NaNO_2$ in food for 7 days (n=5)	48.7±2.6	46.2±5.2	–	–
2b. $NaNO_2$ in food for 14 days (n=4)	–	–	30.8±1.3	19.2±1.3
3a. Treated with PB for 9 days (n=4)	40.4±3.8	37.8±3.8	–	–
3b. Treated with PB for 16 days (n=4)	–	–	28.8±1.9	18.6±1.3
4a. $NaNO_2$ for 7 days + PB for 9 days (n=4)	54.5±4.5*	73.7±5.8*	–	–
4b. $NaNO_2$ for 14 days + PB for 16 days (n=4)	–	–	41.7±2.6*	26.3±2.6

The experimental design is explained under Table 1. * $P < 0.05$ vs controls.

Table 3. Total activity of catalase and acid phosphatase in liver homogenates (mean ± SEM)

Groups	Catalase $\log \frac{So}{S_3} \times \frac{2.3}{3}$ /mg protein	Acid phosphatase µg Pi/mg protein/ 10 min
1. Controls (untreated) (n=9)	5.20±0.097	10.95±0.68
2a. $NaNO_2$ in food for 7 days (n=5)	5.48±0.500	12.13±0.89
2b. $NaNO_2$ in food for 14 days (n=4)	5.16±0.037	11.46±0.31
3a. Treated with PB for 9 days (n=4)	4.60±0.140 §	10.08±0.62
3b. Treated with PB for 16 days (n=4)		8.14±0.36*
4a. $NaNO_2$ for 7 days + PB for 9 days (n=4)	4.47±0.080*	9.03±0.59*
4b. $NaNO_2$ for 14 days + PB for 16 days (n=4)	5.04±0.330	7.74±0.66*

The experimental design is explained under Table 1. S_0 and S_3 – according to Cohen *et al.* (1970); § – average values for days 9 and 16; * $P < 0.05$ vs controls.

and more) (Popova *et al.,* 1982; Popov *et al.,* 1996). In this study, a biphasic effect was established which was manifested by the 14-day treatment. The treatment with PB (groups 3a and 3b), in the shorter term did not change the LP catalyzing systems (group 3a) and inhibited highly only the non-enzymatic system after a prolonged dosing (group 3b). In the groups supplemented with nitrite (groups 4a and 4b), this barbiturate activated statistically significant both systems after a 7-day application and only the enzymatic system after 14-day treatment ($P<0.05$, Table 2).

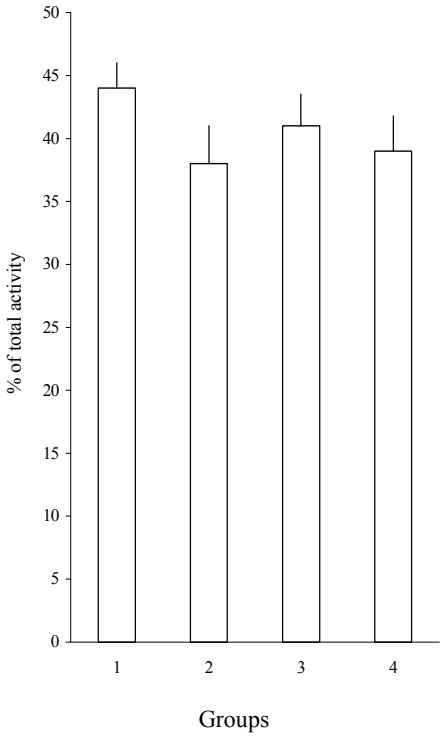

Fig. 1. Nonsedimentable catalase activity in rat liver homogenates (average values); 1– controls (untreated rats given ordinary food) (n=9); 2 – rats that received food containing 0.3 % $NaNO_2$ for 7 and 14 days (n=9); 3 – rats treated with phenobarbital for 9 and 16 days (n=8); 4 – rats that received food with $NaNO_2$ and treated with PB (n=8). The experimental design is explained under Table 1.

Table 3 presents the influence of studied chemicals upon the total liver catalase activity. It could be noticed that PB, applied either separately or in combination with $NaNO_2$ (groups 3 and 4), resulted in enzyme inhibition that was statistically significant in animals that received food with nitrite for a short time (group 4a).

$NaNO_2$ decreased the non-sedimentable catalase activity (Fig. 1) – a sign of peroxisomal stabilization (group 2). A similar effect had PB (group 3), although at a lower extent. When combined with $NaNO_2$, PB did not cause significant redistribution of catalase activity in liver homogenates and therefore, did not change the stability of peroxisomes (group 4).

The total activity of the lysosome marker AP was also affected by tested chemicals. A statistically significant lower acid phosphatase activity was observed in groups, treated 16 days with PB (group 3b) and treated with the combination $NaNO_2$ + PB for either shorter or longer time (groups 4a and 4b) (Table 3).

The chemical agents studied influenced the permeability of lysosome membranes. As shown in Fig. 2, the administration of PB for 9–16 days (group 3) resulted in a high-degree stabilization of liver lysosomes. A similar, but less expressed effect had the treatment with nitrite as well. The effect of $NaNO_2$ predominated during the combined administration of both chemicals despite that the administration of PB preceded that of nitrite and was more prolonged.

The effects of xenobiotics studied were interesting. For example, PB is known as a powerful inductor of enzymes that metabolize toxic substances and drugs (Slater, 1969; Conney, 1972; Pavlova *et al.,* 1978; Popov *et al.,* 1979; Anjum & Shakoori, 1993). In previous studies of

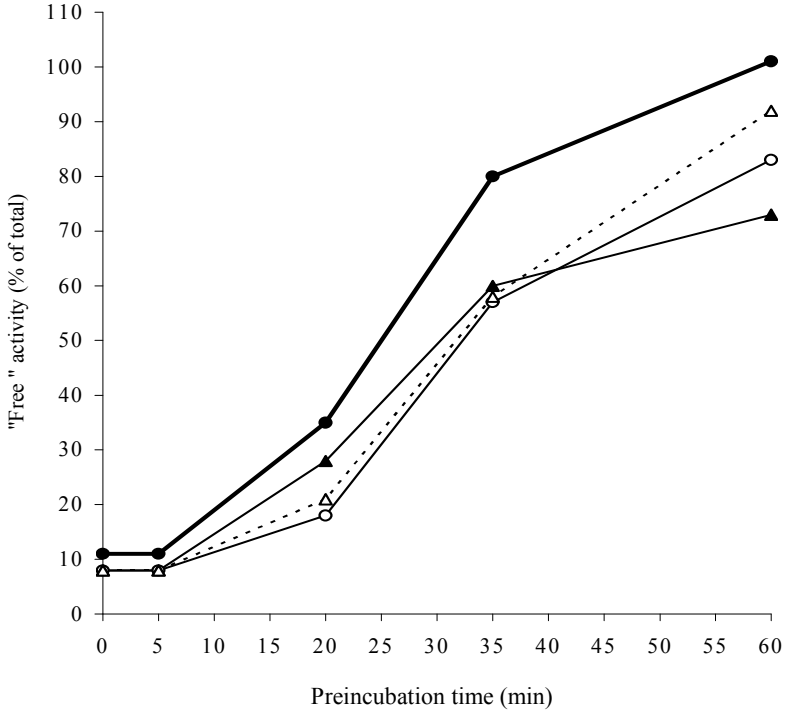

Fig. 2. Release of acid phosphatase (AP) from liver large-granule fractions during their preincubation at pH 5 (0.1M acetate buffer in 0.25 M solution of sucrose) and 37^{O}C. Substrate (Na-β-glycerophosphate) ensuring a final concentration of 0.05 M was added at the end of the preincubation periods and thereafter the incubation was contineued for 10 min. The available enzyme activity is expressed as % to the total once, determined in the presence of Triton X-100 (0.1%). 1 (–•–) controls (untreated rats given ordinary food) (n=9); 2 (–o–) rats that received food containing 0.3 % $NaNO_2$ for 7 and 14 days (n=9); 3 (–▲–) rats treated with phenobarbital for 9 and 16 days (n=8); 4 (--∆--) rats that received food with $NaNO_2$ and treated with PB (n=8).

ours it was found that following a twofold application at 24-hour interval at a dose of 100 mg/kg, it had a stabilizing effect on intracellular membrane structures (Ivanov *et al.,* 1978). The present data suggest that a similar effect was also achieved following a continuous administration of the drug at the same dose (100 mg/kg) (Fig. 2). There was no statistically significant difference in the degree of membrane stabilization attained following 9-day and 16-day treatments. The activating effect of PB on AH however, was different. The activity of this enzyme increased very rapidly after twofold application of PB at 24-h interval (Pavlova *et al.,* 1978) as well as by day 9 of the chronic treatment when the values were several times higher than control ones (Table 1). By day 16 the AH activity was significantly higher vs control values ($P<0.05$) but lower than that by day 9. These data probably suggest that PB induces for a relatively short time a state, known as hypertrophic hypoactive smooth ER (Bunyan & Page, 1978).

The membrane effects and the stimulating action of PB on ER enzymes are influenced by many factors. Usual changes that are observed are deviations in the degree and more rarely – an inversion of effects (Ivanov *et al.,* 1978; Pavlova *et al.,* 1978; Popov *et al.,* 1979). In the present studies, we applied PB as agent creating a background with altered functional ER status in order to elucidate the nitrite effects at this condition. The obtained data (Table 1, Fig. 1 and 2) however showed that rather nitrite had an affect on PB than the contrary. In animals supplemented with nitrite, PB acted as stimulator of LP, its activating effect on AH decreased and its membrane-stabilizing action was completely neutralized. Moreover, a tendency towards increased permeability of lysosome membranes was observed (Fig. 2).

In animal tissues, both nitrates and nitrites are transformed reversibly one into the other by cytosolic and peroxisomal enzymes (Slater, 1969), whereas nitrites are transformed into nitrosamines by enzymes located in the ER (Paul & Illing, 1981). The fact that membrane effects of nitrite were almost not influenced by its co-administration with PB showed that in this connection the activity of the former group of enzymes was more important than that of ER-located enzymes.

CONCLUSIONS

$NaNO_2$, applied separately for a different period of time, provoked a stabilization of peroxisomal membranes and an insignificant activation of AH (by days 7 and 14), of LP (by day 7) and inhibition of LP by day 14 (biphasic effect).

The PB stabilized the membranes of cell organelles studied, activated highly the AH and was almost not producing a change in LP except for the non-enzymatic LP that was inhibited by day 14.

When PB + nitrite were co-administered, the effect of the nitrite predominated on the parameters studied: $NaNO_2$ reduced AH activation, eliminated the stabilization of biomembranes and enchanced the stimulating effect of PB on LP.

REFERENCES

Anjum, F. & A. K. Shakoori, 1993. Effect of cadmium intoxication on drug metabolizing enzymes of promethazine-pretreated and phenobarbitone-pretreated male rabbits. *Pakistan Journal of Zoology,* **25,** 337–346.

Banerje, B. D., V. Seth, A. Bhattacharya, S. T. Paslra & A. K. Chakraborty, 1999. Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicology Letters,* **103,** 33–47.

Boll, M., L. W. D. Weber, E. Becker & A. Stampll, 2001. Pathogenesis of carbon tetrachloride-induced hepatocyte injury. Bioactivation of CCl_4 by cytochrome P-450 and effects on lipid homeostasis. *Zeitschrift für Naturforschung*, **56c**, 111–121.

Bradberry, S. M., B. Gazzard & J. A. Vale, 1994. Methemoglobinemia caused by the accidental contamination of drinking water with sodium nitrite. *Journal of Toxicology – Clinical Toxicology,* **32,** 173–178.

Bruning-Fann, C. S. & J. B. Kaneene, 1993. The effect of nitrate, nitrite and N-nitroso compounds on animal health. *Veterinary and Human Toxicology,* **35,** 237–253.

Bunyan, P. & J. Page, 1978. Polychlorinated biphenyls. The effect of structure on the induction of quail hepatic microsomal enzymes. *Toxicology and Applied Pharmacology,* **43,** 507–518.

Cohen, G., D. Dembiec & J. Marcus, 1970. Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, **30,** 30 – 38.

Conney, A., 1972. Environmental factors influencing drug metabolism. In: *Fundamentals of Drug Metabolism and Drug Disposition*, eds B. La Du, H. Mandel & E. Way, The William a. Wilkins Co., Baltimore, pp. 253–278.

Dhawan, D. K. & A. Goel, 1994. Protective role of zinc on rat liver function on long term toxicity induced by carbon tetrachloride. *Journal of Trace Elements in Experimental Medicine*, **7,** 1–9.

Elves, R. G., T. H. Ueng & A. P. Alvares, 1985. Regulation of hepatic monooxygenases by phenobarbital, 3-methylcholantrene and polychlorinated biphenyl in rapid and slow acetylator mice. *Drug Metabolism and Dispositition*, **13,** 354–358.

Gianetto, R. & C. De Duve, 1955. Tissue fractionation studies. 4. Comparative study of the binding of acid phosphatase, b-glucuronidase and cathepsin by rat liver particles. *Biochemical Journal,* **59,** 433 – 438.

Griffin, J. P., 1997. Methemoglobinemia. *Adverse Drug Reactions and Toxicological Reviews,* **16,** 45–63.

He, Q. & X. Wang, 1995. Effect of CCl_4 on Cd-induced renal damage. *Journal of Toxicology – Clinical Toxicology,* **33,** 541–542.

Hodgson, E., R. L. Rose, D. Y. Ryu, G. Falls, B. L. Blake & P. E. Levi, 1995. Pesticide-metaboliting enzymes. *Toxicology Letters*, **82–83,** 73–81.

Ivanov, N., M. Pavlova & Ch. Popov, 1978. Stability of liver cell organelles as affected by nutrition regime and chemical agents. *Animal Science,* **15,** No 2, 81–88 (BG).

Koleva, M., A. Kastelova, D. Staneva-Stoytcheva & Ts. Stoytchev, 1999. Combined effect of propranolol with nifedipine or with diltiazem on rat liver monooxigenase activities. *Toxicology Letters*, **105,** 153–161.

Langlois, C. J. & E. J. Calabrese, 1992. The interactive effect of chlorine, copper and nitrite on methemoglobin formation in red blood cells of Dorset sheep. *Human and Experimental Toxicology,* **11,** 223–228.

Liu, Y., D. P. Hartley & J. Liu, 1998. Protection against carbon tetrachloride hepatotoxicity by oleanolic acid is not mediated through metallothionein. *Toxicology Letters*, **95,** 77–85.

Madhukar, B. V. & F. Matsumura, 1981. Differences in the nature of induction of mixed-function oxidase systems of the rat liver among phenobarbital, DDT, 3-methylcholantren and TCDD. *Toxicology and Applied Pharmacology*, **61,** 109–118.

Mannering, G. J., 1972. Microsomal enzyme system which catalize drug metabolism. In: *Fundamentals of Drug Metabolism and Drug Disposition*, eds B. La Du, H. Mandel & E. Way, The William a.Wilkins Co., Baltimore, pp. 206–252.

Meyer, U. A. & K. Hoffmann, 1999. Phenobarbital-mediated changes in gene expression in the liver. *Drug Metabolism Reviews*, **31,** 365–373.

Mochizuki, H., J. Takido & H. Yokogoshi, 1999. Effect of dietary taurine on endogenous hypercholesterolemia in rats fed on phenobarbital-containing diet. *Biosciences Biotechnology and Biochemistry,* **63,** 1298–1300.

Paul, H. & A. Illing, 1981. Does the cytochrome P-450 dependent mixed function oxigenase participate in nitro group reduction in liver? *Biochemical Society Transactions*, **9,** 268–272.

Pavlov, D., Y. Staykov, T. Mircheva, J. Mitev & D. Djouvinov, 1999. Influence of nitrates on the growth of tillapia and possibilities for reducing their detrimental effect. *Bulgarian Journal of Agricultural Science*, **5,** 449–452.

Pavlova, M., Ch. S. Popov & N. Ivanov,1978. Effect of diet on changes in the activity of some enzymes located in the endoplasmic reticulum, caused by chemical agents. *Comptes rendus de l'Académie bulgare des Sciences*, **31,** 101–104.

Popov, Ch. S., 1972. Effect of chlorpromazine on the stability of rat liver mitochondria,

lysosomes and peroxisomes. *Comptes rendus de l'Académie bulgare des Sciences*, **25,** 1293–1296.

Popov, Ch. S., 1976. Effect of certain stress factors on the activity of some enzymes and enzyme systems of the endoplasmic reticulum. *Comptes rendus de l'Académie bulgare des Sciences*, **29,** 715–718.

Popov, Ch. S., I. Yantchev, G. Georgiev & L. Geneva, 1976. Effect of different factors modifying the activity of some enzyme systems of the endoplasmic reticulum on the sensitivity of cell organelles against the damaging action of chemical agents. I. Interrelations between the activity of some enzyme systems located in endoplasmic reticulum and hepatotoxicity of carbon tetrachloride. *British Journal of Experimental Pathology,* **57,** 593–603.

Popov, Ch. S., I. Yantchev, M. P. Popova & G. M. Vulcheva, 1979. Effect of different factors modifying the activity of some enzyme systems of the endoplasmic reticulum on the sensitivity of cell organelles against the damaging action of chemical agents. II. Studies with chlorpromazine, 2,4-dinitrophenol, phenobarbital and DDT. *British Journal of Experimental Pathology,* **60,** 85–95.

Popov, Ch. S., Y. N. Georgiev, M. P. Popova & M. D. Mironova, 1982. Changes in the stability of liver lysosomes and peroxisomes produced by nitrates and nitrites. *Comptes rendus de l'Académie bulgare des Sciences*, **35,** 93–96.

Popov, Ch. S., M. P. Popova & M. D. Mironova, 1990. Influence of some xenobiotics on the aerobic glycolysis and superoxide production by rat peritoneal polymorphonuclear leucocytes. *Comptes rendus de l'Académie bulgare des Sciences*, **43,** 137–140.

Popov, Ch., D. Pavlov, M. Popova, R. Gahniyan, A. Ilchev & I. Bozadjiev, 1996. Influence of sodium nitrate applied separately and in combination with other compounds on blood and tissue parameters of rats and wethers. *Bulgarian Journal of Agricultural Science,* **2,** 405–412.

Popova, M. P., Ch. S. Popov, Y. G. Nikolov & M. D. Mironova, 1982. Influence of nitrates and nitrites on the activity of enzymes located in the endoplasmic reticulum. *Comptes rendus de l'Académie bulgare des Sciences,* **35,** 961–969.

Popova, M., Ch. Popov, D. Pavlov & Y. Staykov, 1999. Changes in some fish blood parameters produced by sodium and potassium nitrate mixture and by its combination with artichoke extract. *Bulgarian Journal of Agricultural Science,* **5,** 945–948.

Popova, M. P. & C. S. Popov, 2002. Damage to subcellular structures evoked by lipid peroxidation. *Zeitschrift für Naturforschung,* **57c,** 361–365.

Popova, M., 2004. Diet and effect of $NaNO_2$ on some functional activities of rat liver endoplasmic reticulum. *Animal Science,* **41,** 42–45 (BG).

Slater, T. F., 1969. Lysosomes and experimentally induced tissue injury. In: *Lysosomes in Biology and Pathology*, eds J. T. Dingle & H. B. Fell, North-Holland Publ.Co., Amsterdam. London, **1,** pp. 467–492.

Wills, E. D., 1969. Lipid peroxide formation in microsomes, general considerations. *Biochemical Journal,* **113,** 315–327.

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