Some Mechanisms of Toxic Action of Irritants

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SOME MECHANISMS OF TOXIC ACTION OF IRRITANTS

The influence of 2-chlorobenzylidene malononitrile (CBM) and dibenz[b,f]-1,4-oxazepine (DBO) on the acid-base balance state in the blood, including on a background of nitrite of sodium (NS) and thiosulfate of sodium (TS) and activity of cytochrom-c-oxidase (CHO) in the homogenate of liver tissue of rats was examined . It was used intraperitoneal and aerogenous introduction of irritants in doses 0,5 LD_{50}, LD_{50} and LCt_{50}. Obtained results of experiments show that intoxication with CBM and DBO is characterised by violation of tissue respiration which results in the state of metabolic acidosis. Oppressive effect of CBM on activity of CHO was observed. DBO doesn't have influence of inhibition on CHO activity.

1.0 INTRODUCTION

Influence of irritating agents (IA) cause temporary incapacitation of people [1, 3, 4]. Using of IA for military purposes is possible in situations of collision with terrorists, stopping or preventing reconnaissance-diversionary actions, law-enforcement, personal self-defense aims and ctr. Taking into consideration, that at creation of new, more effective irritating wares, the basic requirement must be harmlessness for the health of their active components, we studied displays of resorptive action of most widespread IA: 2-chlorobenzylidene malononitrile (CBM) and dibenz[b,f]-1,4-oxazepine (DBO) [2, 5].
For providing of vital processes, pretty strict constancy of organism liquids, the integral index of which is acid-base balance of blood, is requires. We examined influence of CBM and DBO on the acid-base balance state and activity of cytochrom-C-oxidase (CHO).

2.0 METHODS EMPLOYED

Influence of CBM and DBO on the acid-base balance state was studied on rats. The mixed blood of intact animals, animals exposed to influence of these agents and also on a background of nitrite of sodium and thiosulfate of sodium, used as pharmacological analyzers, was explored. It was used intraperitoneal introduction of irritants in doses 0,5 LD_{50}, LD_{50} and aerogenous introduction in LCt_{50} (proper for mouse). Blood sampling was made by decapitation after 5, 15, 30, 60, 120 and 240 min. after intoxication. The blood collected into glass test-tubes with heparin under liquid petrolatum. Male and female rats by mass of 180-220 g were used in experiments; experimental groups included 6 - 10 animals.
The state of acid-base balance evaluated by such indexes [6] as: actual reaction of blood - pH, the deficit of buffer basements - DBB, the buffer basements - BB, the standard bicarbonate - SB, the veritable bicarbonate — VB, partial and total tensions of carbonic acid – PCO_2 and TCO_2, and also partial tension of oxygen - PO_2.
Determination of activity of CHO was made in homogenate of rats' livers tissue [7, 8]. The studied irritants were introduced intraperitoneally: CBM in doses 0,5 LD50 and LD50, and DBO - in the dose of LD50. As a control the livers tissue of intact rats was used (intraperitoneal introduction of solvent). Sapling of the explored material was made in the case of CBM through 15, 30, 60 min., and in the case of DBO - through 30 min. after introduction.

Levchenko, O.E.; Mokhort, N.A. (2007) Some Mechanisms of Toxic Action of Irritants. In *Defence against the Effects of Chemical Hazards: Toxicology, Diagnosis and Medical Countermeasures* (pp. 21-1 – 21-12). Meeting Proceedings RTO-MP-HFM-149, Paper 21. Neuilly-sur-Seine, France: RTO. Available from: http://www.rto.nato.int.

3.0 RESULTS OBTAINED

Intraperitoneal introduction of CBM in dose 0,5 LD_{50} (Fig 1, 2, 3) (15mg/kg) caused after 5 min. substantial changes of acid-base balance comparatively to the indexes of controls. They were expressed in the reliable increase of blood pH, reduction of VB, PCO_2 and TCO_2. There was also a tendency to increasing of DBB, and decreasing of BB, SB. At the same time PO_2 increased sharply. So, in an organism in this case the state of compensated metabolic acidosis develops in combination with gas alkalosis.

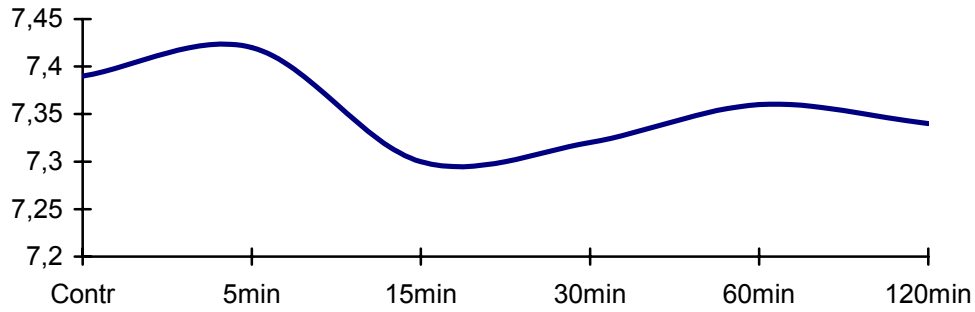

Figure 1: pH of rats' blood after intraperitoneal introduction of CBM in dose 0,5 LD50

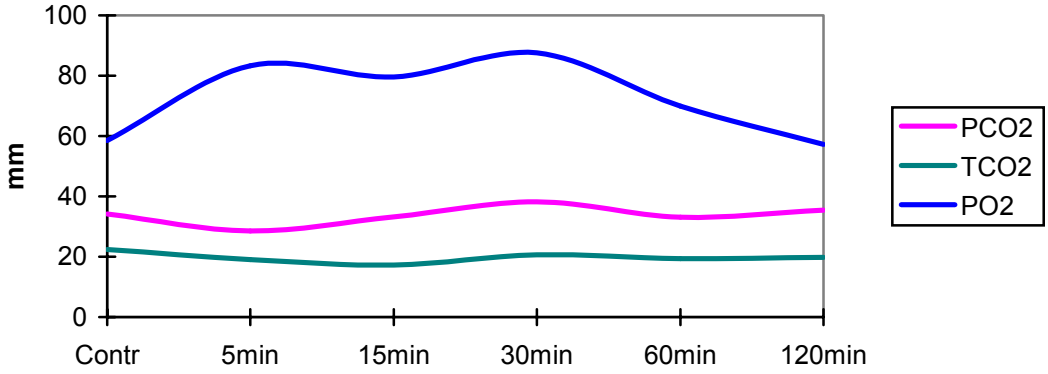

Figure 2: PCO_2, TCO_2, PO_2 of rats' blood after intraperitoneal introduction of CBM in dose 0,5 LD50

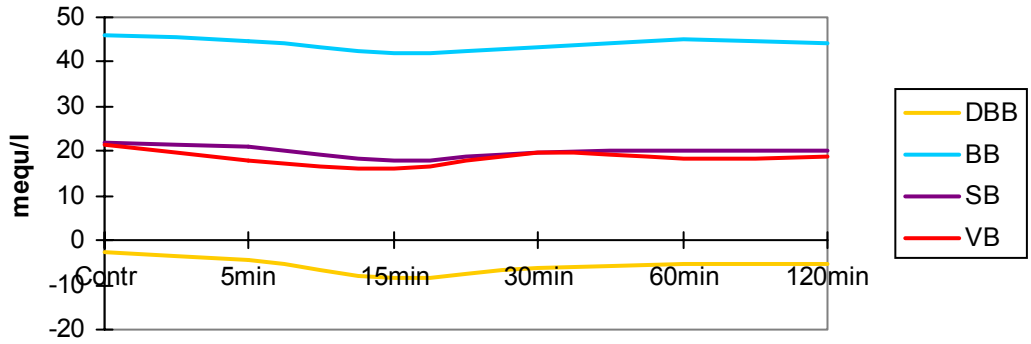

Figure 3: DBB, BB, SB, VB of rats' blood after intraperitoneal introduction of CBM in dose 0,5 LD50

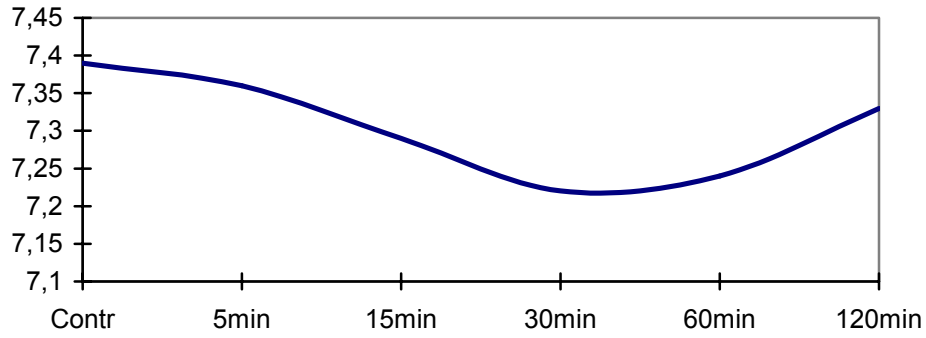

Figure 4: pH of rats' blood after intraperitoneal introduction of CBM in dose LD50

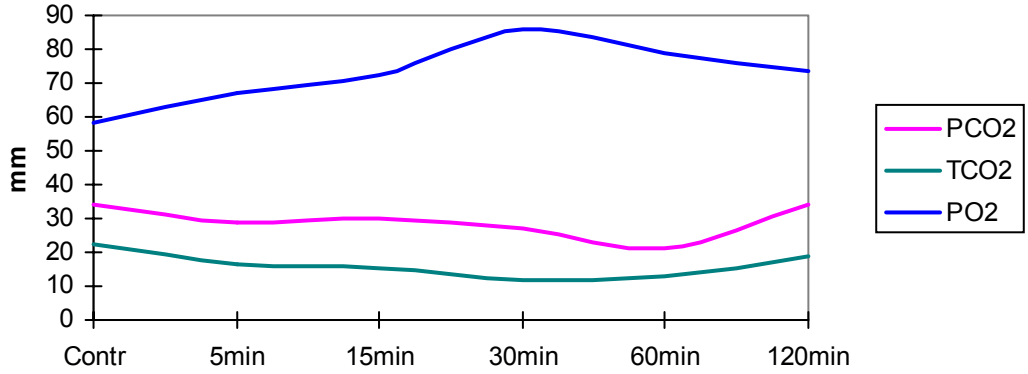

Figure 5: PCO_2, TCO_2, PO_2 of rats' blood after intraperitoneal introduction of CBM in dose LD50

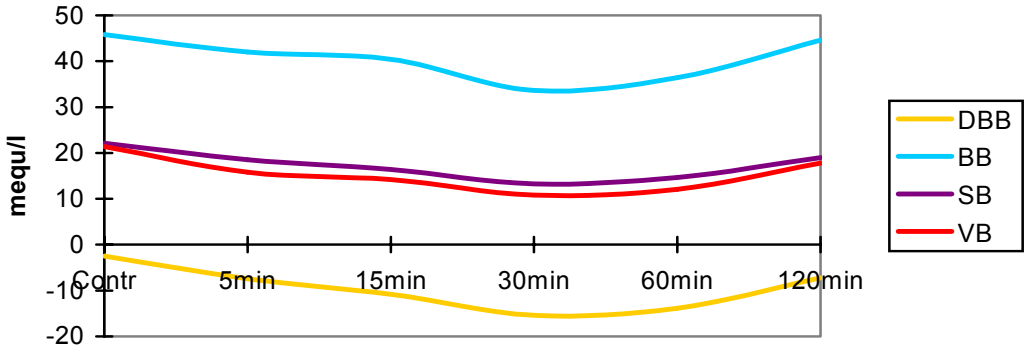

Figure 6: DBB, BB, SB, VB of rats' blood after intraperitoneal introduction of CBM in dose LD50

After 15 min. the state of expressed subcompensated acidosis was developed: the pH of blood went down, the DBB began to grow, the BB, SB, VB diminished more, PCO_2 and TCO_2 went down also. PO_2 remained increased.

Compensated mechanisms, probably, are manifistated after 30 min., multiplying of carbonic acid partial tension testifies to that. At the same time, the state of compensated metabolic acidosis is saved, that is confirmed by the low level of blood pH, high DBB, low value of TCO_2. PO_2 as compared to control in this period of supervision increased almost on 50%.

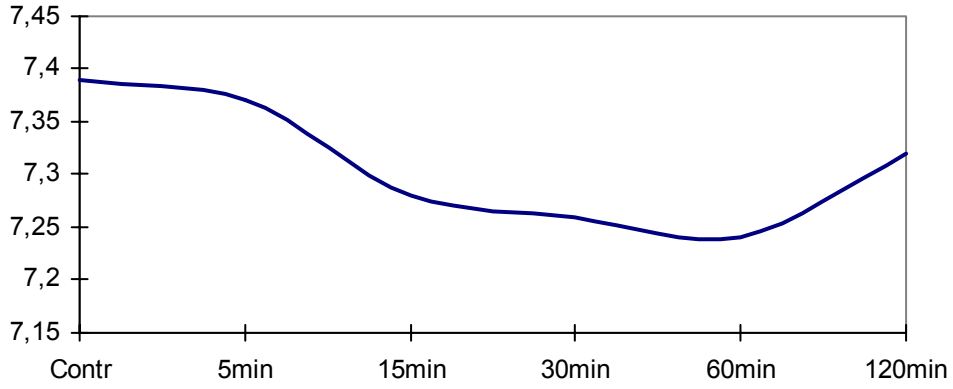

Figure 7: pH of rats' blood after intraperitoneal introduction of DBO in dose 0,5 LD50

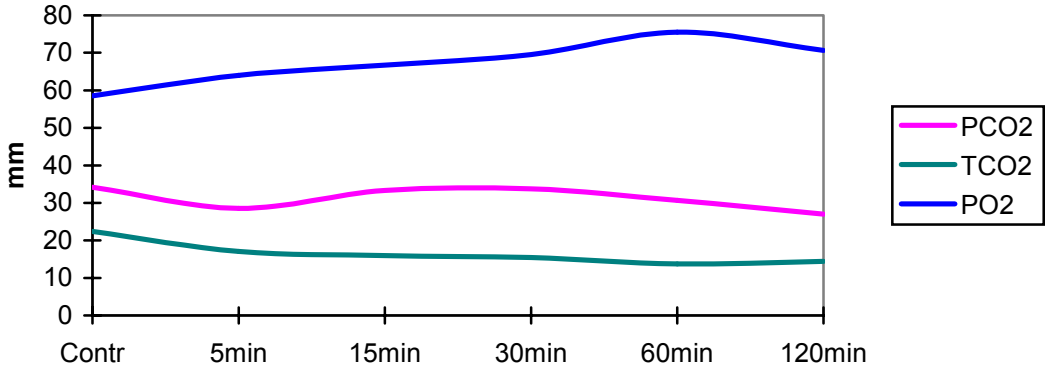

Figure 8: PCO_2, TCO_2, PO_2 of rats' blood after intraperitoneal introduction of DBO in dose 0,5 LD50

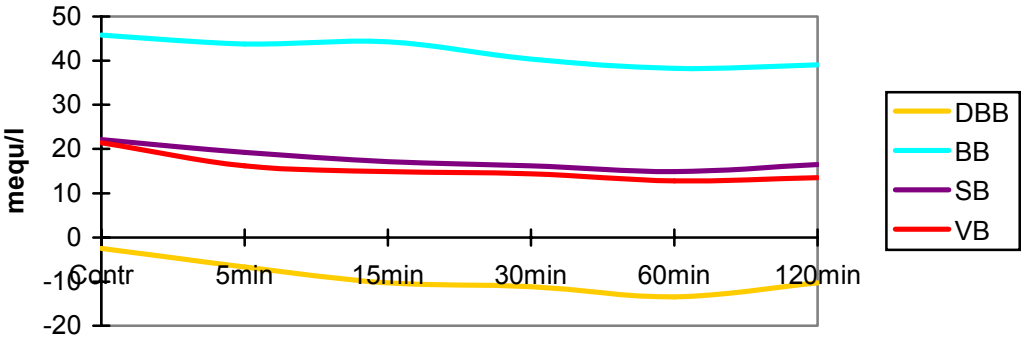

Figure 9: DBB, BB, SB, VB of rats' blood after intraperitoneal introduction of DBO in dose 0,5 LD50

Close to above-described were indexes of acid-base balance of blood after 60 and 120 min. However, the pH of blood to this time acquires a tendency to normalization, and PO_2 in blood by 120 min. is normalized. After intraperitoneal introduction in dose of LD_{50} CBM (Fig 4, 5, 6) caused more considerable and significant changes of the acid-base balance as compared to dose of 0,5 LD_{50}. So, after 5 min. the indexes of acid-base balance changed on a type to characteristic for the metabolic acidosis state. All changes, except for PCO_2, were significant, that testifies to more substantial violations.

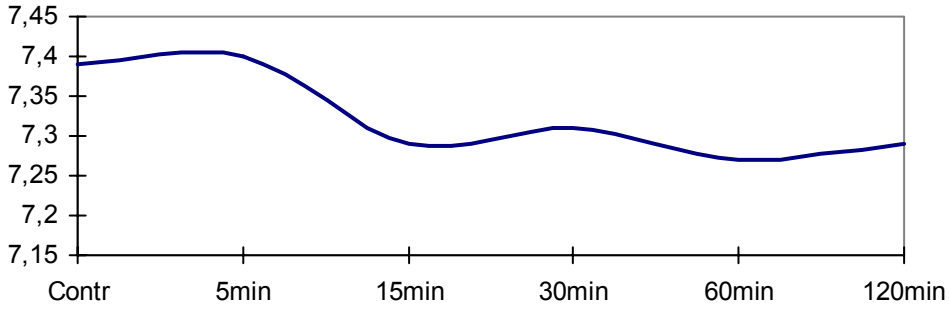

Figure 10: pH of rats' blood after intraperitoneal introduction of DBO in dose LD50

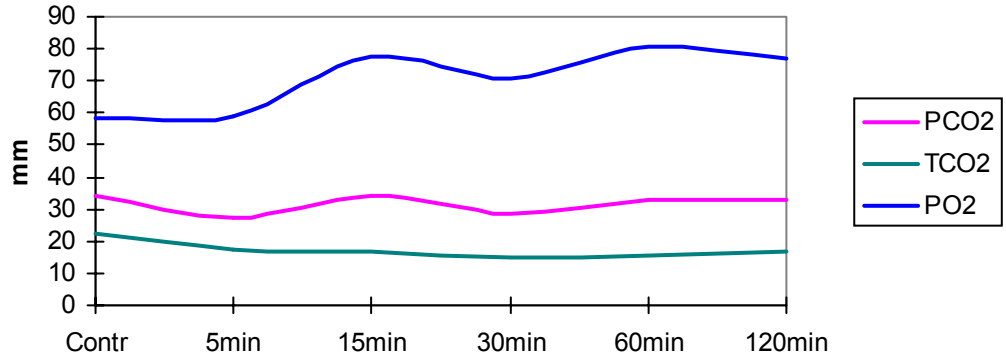

Figure 11: PCO_2, TCO_2, PO_2 of rats' blood after intraperitoneal introduction of DBO in dose LD50

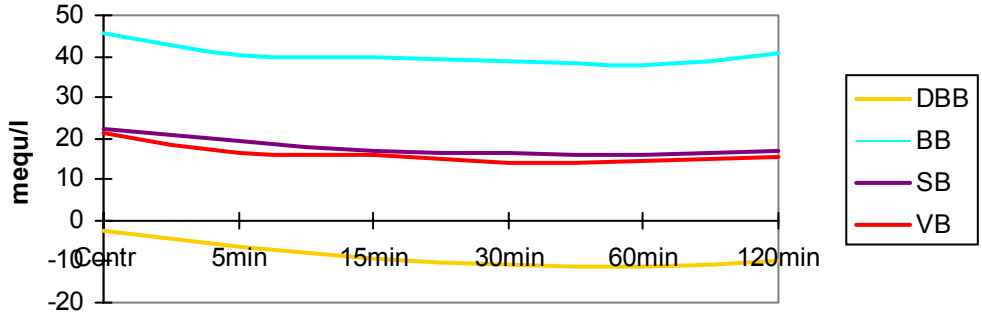

Figure 12: DBB, BB, SB, VB of rats' blood after intraperitoneal introduction of DBO in dose LD50

After 30 min. changes on the type of decompensated acidosis were observed. As compared to initial indexes levels of pH, BB, SB, VB, PCO_2 and TCO_2 fell down. The DBB increased, and PO_2 grew.
After 2-4 hours after introduction there was a tendency to normalization of acid-base balance. However, if to compare the changes of the acid-base state, arising up as a result of CBM actions in different doses, evidently, that at using of this matter in the dose of LD_{50} they remain more substantial.Thus, the results of the above-mentioned researches showed that the degree of violation of parameters of acid-base balance at intraperitoneal introduction of CBM had depended on the applied dose of the last.

Similar experiments were done at studies of toxic action of DBO. At intraperitoneal introduction in a dose 0,5 LD_{50} (Fig 7, 8, 9) (237,5mg/kg) after 5 min. also there was the state of metabolic acidosis in combination with gas alkalosis. All changes, unlike such in this period at application of CBM were significant and more expressed. At the same time PO_2 in blood was not increased reliably.
In subsequent periods of researches the state of acid-base balance of animals was worsened. The most substantial changes of indexes, characterizing the state of decompensated metabolic acidosis were observed after 60 min. after influence. As compared to the indexes of controls, for certain went down pH of blood, BB, SB, VB, PCO_2 and TCO_2 . DBB grew More than in 5 times. To this time PO_2 was considerably increased.
A tendency to renewal of indexes of acid-base balance was observed in 2-4 hours after introduction and although they were yet distant to a norm, however already considerably differed from such, as compared to 60 min. of experiment. PO_2 , as compared to basic data remained high.
Intraperitoneal introduction of DBO in the dose of LD_{50} caused (Fig 10, 11, 12) changes, similar to its action in a dose of 0,5 LD_{50}.
So, in 5 min. after introduction of DBO in the dose of LD_{50} the state of compensated metabolic acidosis developed in combination with gas alkalosis. Parameters of acid-base balance were near to such at the action of dose 0,5 LD_{50}, all of them changed significantly by comparison to the indexes of controls. PO_2 in blood here practically did not change.

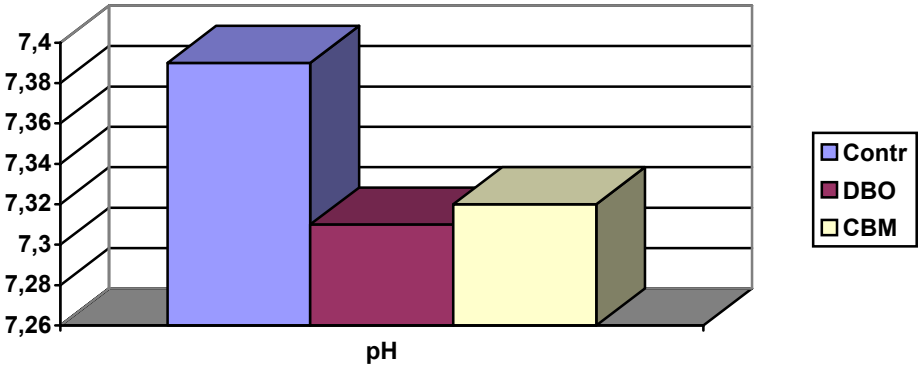

Figure 13: pH of rats' blood after inhalation of DBO and CBM (LCt_{50})

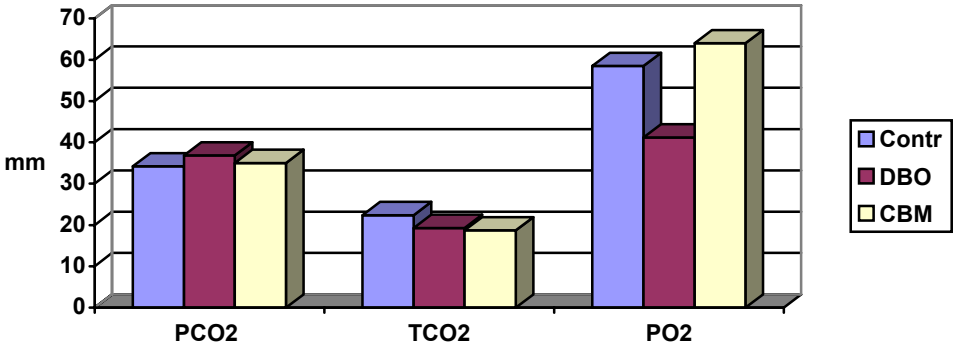

Figure 14: PCO_2, TCO_2, PO_2 of rats' blood after inhalation of DBO and CBM (LCt_{50})

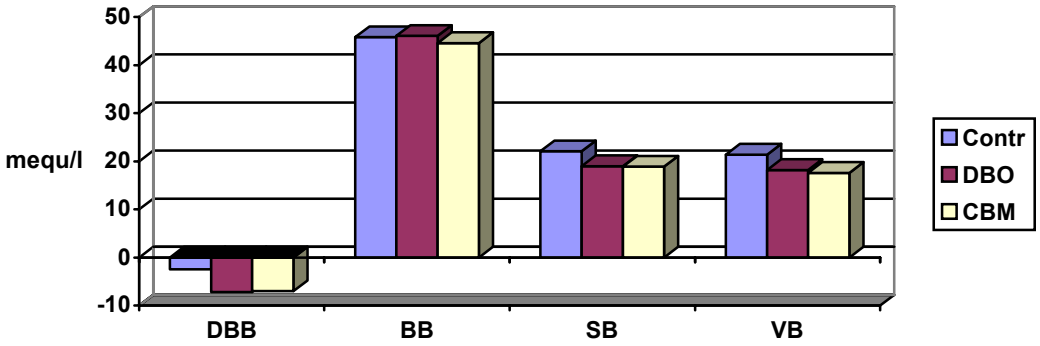

Figure 15: DBB, BB, SB, VB of rats' blood after inhalation of DBO and CBM (LCt_{50})

Most substantial change of acid-base balance observed also after 60 min. As compared to the values of controls the pH of blood, BB, SB, VB, PCO_2 and TCO_2 significantly went down . The DBB and PO_2 was increased.

Tendency to normalization of acid-base balance at the action of DBO in the dose of LD_{50} as well as at introduction of dose 0,5 LD_{50}, observed after 120 min. after entering of matter into organism.

All described violations are characteristic for the state of decompensated metabolic acidosis and it is not found out dependences of degree of their changes on the operating dose of DBO.

Thus, as a result of the conducted researches it is set, that the degree of violations of constancy of internal environment, arising up as a result of intoxication of CBM depended on the size of acting dose, at the same time it is not discovered for DBO of such dependence. It should be noted that the doses of CBM and DBO compared in equivalent correlation differed between itself in number almost in 17 times. Nevertheless, DBO and CBM caused changes of the state of acid-base balance near by the values.

At the use for introduction of the most credible way of entering organism of irritants — inhalation, animals were exposed to influence of CBM and DBO in doses, proper LCt_{50} for mouse (53,0 and 76,0 mg·min/l accordingly). Blood was explored after 30 min. after introduction. Obtained results (Fig 13, 14, 15) testify that both irritants cause development of subcompensated metabolic acidosis. To the significant changes the pH, DBB, SB and VB, TCO_2 undergoes.

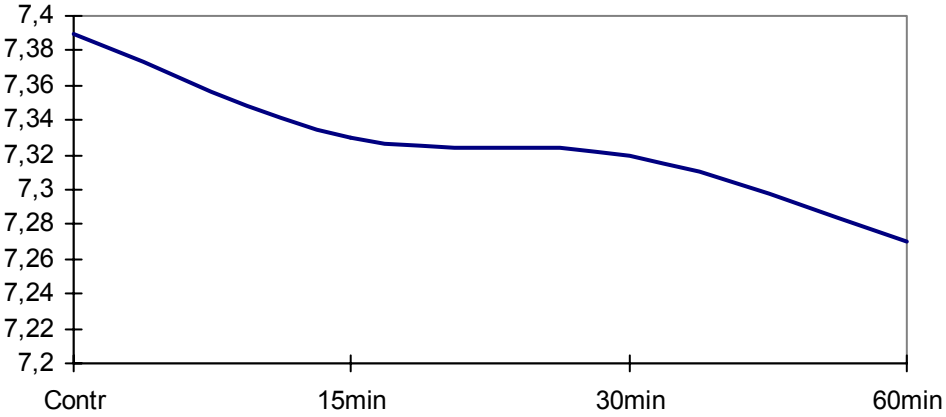

Figure 16: pH of rats' blood at intraperitoneal introduction of CBM (LD_{50}) after NS

Development of this pathology at introduction of CBM it is possible to connect with the action of cyan-ion, freeing oneself in the process of metabolism. It was of interest to find out influence of these irritants on acid-base balance at introduction them after the pharmacological analyzers: nitrite of sodium (NS) and thiosulfate of sodium (TS), being antidotes at the cyanides poisonings.

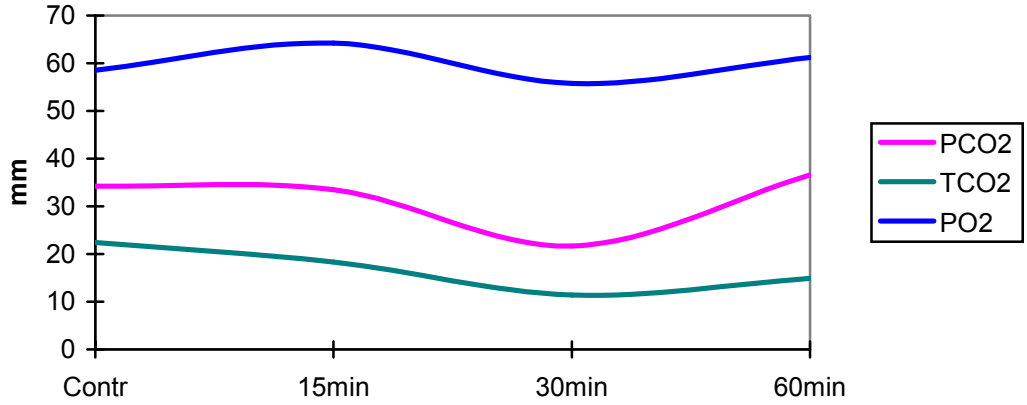

Figure 17: PCO_2, TCO_2, PO_2 at intraperitoneal introduction of CBM (LD_{50}) after NS

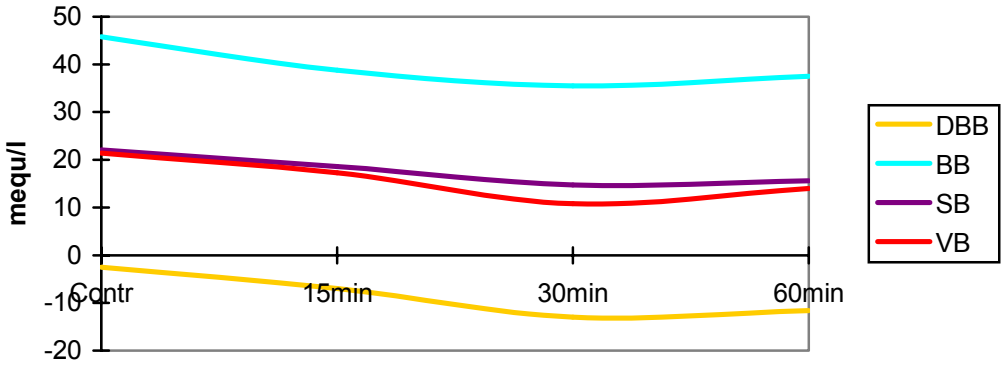

Figure 18: DBB, BB, SB, VB at intraperitoneal introduction of CBM (LD_{50}) after NS

The NS (24 mg/kg) and TS (500 mg/kg*)* introduced intramuscular 10 min. before the intraperitoneal injection of irritants in the doses of LD_{50}.

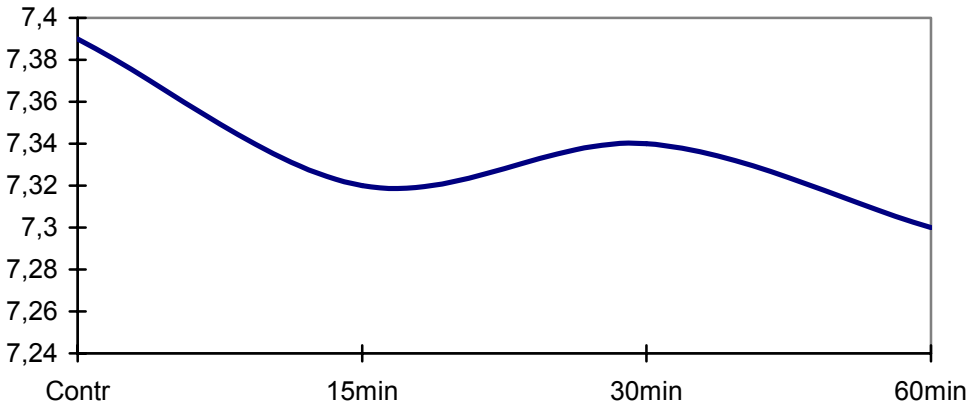

Figure 19: pH of rats' blood at intraperitoneal introduction of DBO (LD_{50}) after NS

Using of NS certainly prevented changes of acid-base balance caused by CBM introduction (Fig 16, 17, 18).

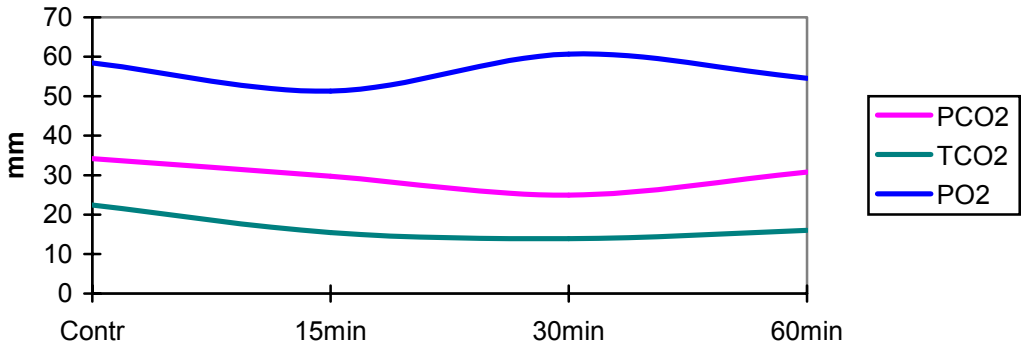

Figure 20: PCO_2, TCO_2, PO_2 at intraperitoneal introduction of DBO (LD_{50}) after NS

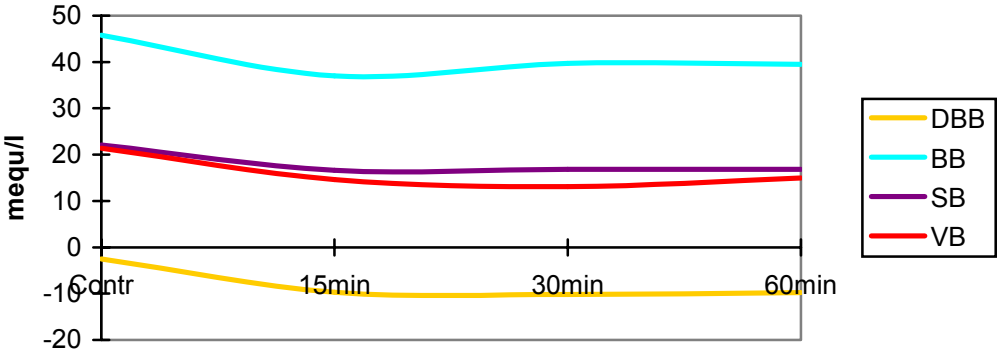

Figure 21: DBB, BB, SB, VB at intraperitoneal introduction of DBO (LD_{50}) after NS

So, through 15 min there were significant changes of basic indexes, characterizing the state of metabolic acidosis (pH, SB, VB) toward normalization, by comparison to the untreated group of animals. There was also a tendency to normalization of other indexes: PCO_2 and TCO_2, DBB, PO_2.
After 30 min. significantly changed pH of blood, and after 60 min.- PCO_2 . Other parameters acquired a tendency to normalization. PO_2 in these time intervals normalized fully.

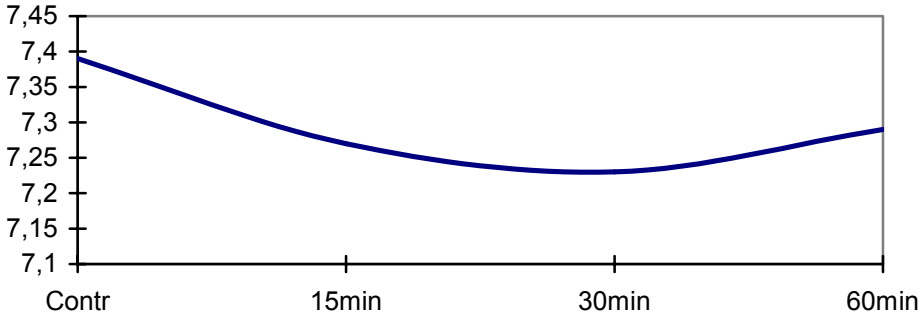

Figure 22: pH of rats' blood at intraperitoneal introduction of CBM (LD_{50}) after TS

Preliminary introduction of NS in animals poisoned by DBO practically did not have normalizing influence on the acid-base balance (Fig 19, 20, 21). Some tendency to renewal was observed only for the Ph of blood.

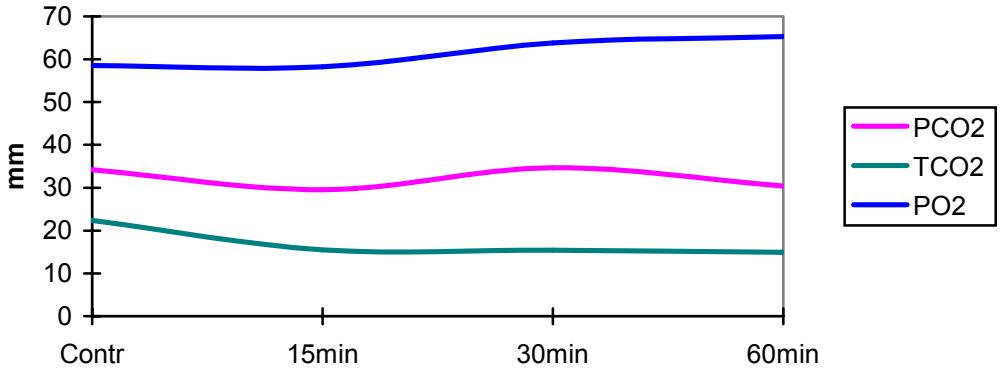

Figure 23: PCO_2, TCO_2, PO_2 of rats' blood at intraperitoneal introduction of CBM (LD_{50}) after TS

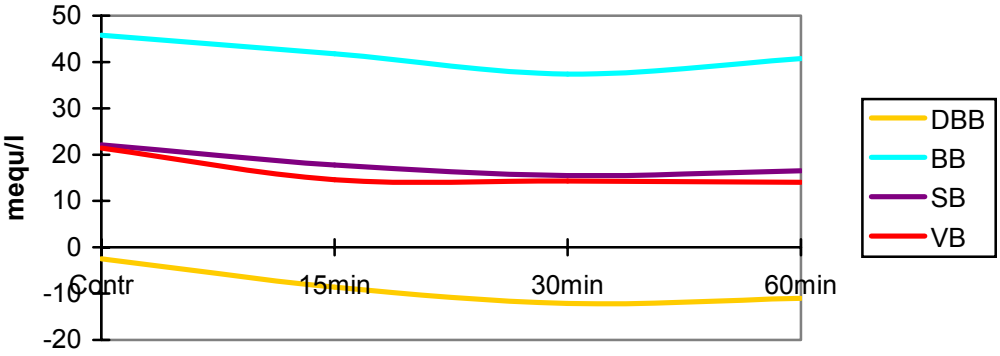

Figure 24: DBB, BB, SB, VB of rats' blood at intraperitoneal introduction of CBM (LD_{50}) after TS

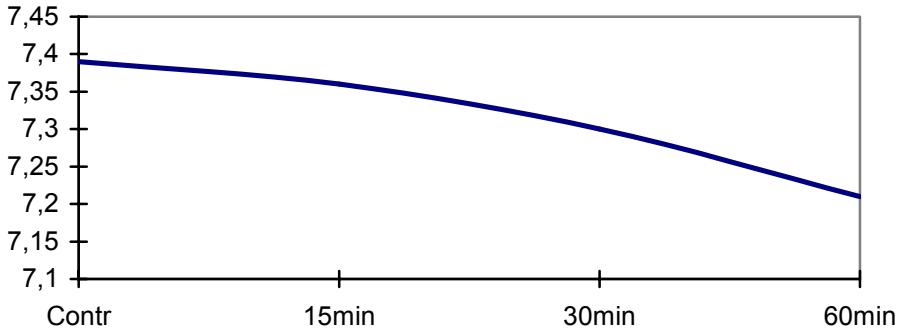

Figure 25: pH of rats' blood at intraperitoneal introduction of DBO (LD_{50}) after TS

The significant normalizing effect of NS showed up only in regard to PO_2.
TS practically did not prevent development of metabolic acidosis at an action both CBM (Fig 22, 23, 24) and DBO (Fig 25, 26, 27). However, there was reliable normalization of PO_2 in both cases.
Thus, the results of the above-mentioned researches testify that at a hit in the organism of toxic doses of explored irritants there is a deep change of constancy of internal environment, expressed in the states of different degree of metabolic acidosis. Development of similar pathology is a consequence of violation of processes of the tissue breathing. Biochemical mechanism of toxic action of CBM, probably, related to the action of cyan-ion, freeing oneself in the process of his metabolism. The toxic influencing of cyanides is taken mainly by binding of cyan-ion to oxidized form CHO, here complex connection appears with its

iron. Such interaction deprives the enzyme of catalytic function in the reaction of aerobic oxidization of cytochrom-C. This results in violation of the tissue breathing and formation of energy in a cell.

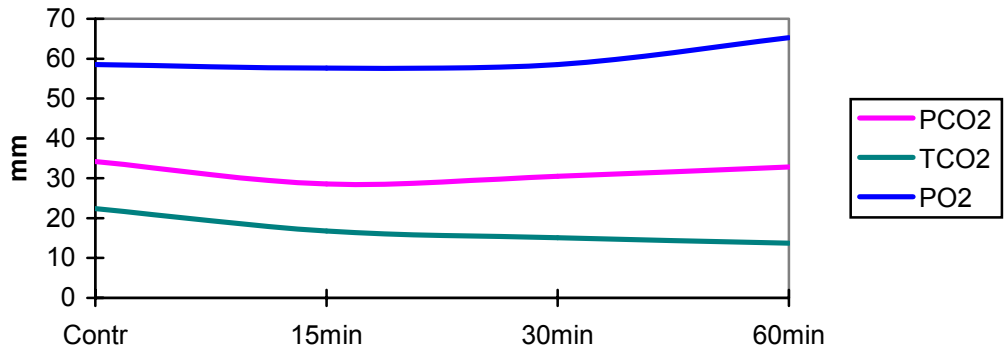

Figure 26: PCO_2, TCO_2, PO_2 of rats' blood at intraperitoneal introduction of DBO (LD_{50}) after TS

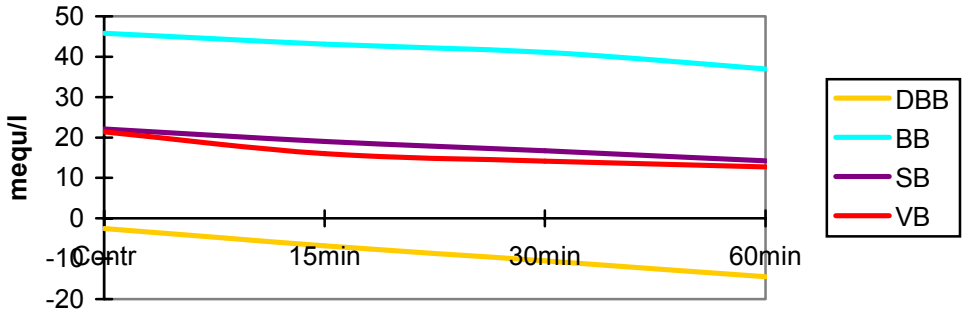

Figure 27: DBB, BB, SB, VB of rats' blood at intraperitoneal introduction of DBO (LD_{50}) after TS

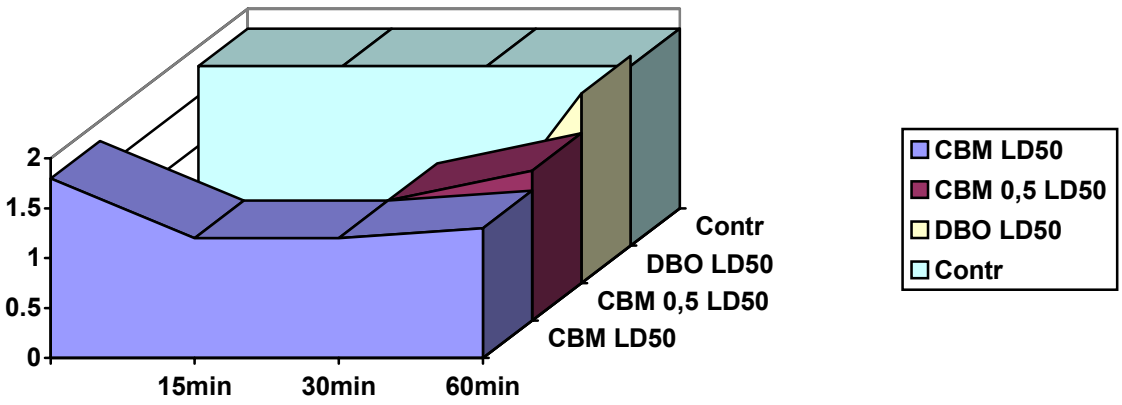

Figure 28: Influence of CBM and DBO on activity of CHO

With violation of breathing process oxidative phosphorylation is violated, that stimulates formation of energy on a pentose and phosphate way. As a result of such reactions there is an accumulation of unoxidated products in an organism — lactic acid, acetone bodies and ctr. Because of accumulation of these intermediate products of metabolism the state of metabolic acidosis arises.
Taking into account the orientation of toxic action of CBM, it was expedient to study influence of explored irritants on activity of CHO.
Results obtained testify to considerable oppressive influence of CBM on CHO activity (Fig 28). It is set that through 30 min. after introduction of CBM in a dose of 0,5 LD_{50} activity of the enzyme was oppressed. After 1 hour there was already a process of renewal of activity, but oppressing was saved. At research of CBM in the dose of LD_{50} observed the same degree of enzyme inhibition after 15 min. and it was saved more long time.
Unlike CBM, at intraperitoneal introduction of DBO in the dose of LD_{50} (475 mg/kg) it practically did not have influence on activity of CHO.

4.0 CONCLUSIONS

1. Pathogenesis of intoxication of both irritants CBM and DBO is characterised with violation of tissue respiration which results in the state of metabolic acidosis.
2. Mechanisms of CBM affection are connected with suppression of activity of cytochrom-C-oxidase. DBO doesn't have influence of inhibition on cytochrom-C-oxidase activity.
3. The degree of violations of constancy of internal environment, arising up as a result of intoxication of CBM depended on the size of acting dose, at the same time it is not discovered for DBO of such dependence.
4. Antidotes of cyan-ion could be effective in treatment in cases of poisoning by CBM.

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