THE EFFECT OF EXPERIMENTAL CHLORINE POISONING ON CERTAIN AMINO ACIDS AND ENZYMES IN RATS, FED ON RICH PROTEIN RATION

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Although acute occupational chlorine poisonings have been thoroughly investigated, the problem of chronic intoxications is still not fully clarified. The latter assumes great importance under the acutal conditions of steadily expanding industrial production and utilization of chlorine in various spheres of present material living conditions.

There is still insufficient data in the pertinent literature, concerning the clinical picture and changes in metabolite-catabolite processes during chronic chlorine influencing. The increase of the non-specific total morbidity rate of the respiratory system among persons engaged in chlorine production is beyond any doubt. Some humoral changes are also recorded, such as reduction of the alkaline reserve and catalase of the blood, rise of bilirubin serum level etc.

The organic and functional alterations observed are attributed to tissue destruction, subsequent to dehydrogenation of the tissue fluids. The more intimate effects, exerted upon the metabolic processes, respectively, the quantitative and qualitative changes in protein and amino acid content, are not fully clarified. The data available about the activity of the enzymes within the various biological substrates in chlorine poisoning are even less complete.

The interest in the study of pathogenetic changes under the aspect thus outlined is justified by the necessity of discovering new biochemical tests for early diagnosis of the chronic effects of chlorine.

On the other hand, the problem of prophylactical occupational nutrition of chlorine contact workers also awaits solution. After demonstrating the tissue-destructive action of chlorine, the question is posed about exogenous influencing, without underestimating the limitations existing in this respect, of the protein — amino acid exchange by means of differentiated prophylactic and therapeutic-prophylactic nutritive diets in chronic effects of chlorine.

The literature data and personal experience alike (1, 2, 3, 4, 5, 11) prove the significance of proteins as a protective factor in the occurrence and development of occupational intoxications. This effect is based on the quantitative and qualitative composition of the administered proteins. Insofar as chlorine is concerned, there are no investigations in this direction, except for the Soviet nutrituve ration No. 2 already adopted.

Table 1

No. of Group	Ration	Controls	Poisoned a single time	Poisoned for 5 days
I	Fed on ordinary laboratory diet (18% cal protein)	10	10	15
II	Fed on rich protein ration (38% cal protein)	10	15	—

Table 2

Changes in the Free Amino Acids Content

	Amino acids	Poisoned onefold				5-day poisoning	
		Ration				Ration	
		I Control	Poisoned	II Control	Poisoned	I Control	Poisoned
Brain	cys	4,38±1,05	3,25±0,72	5,23±1,12	3,29±1,00	4,60±1,02	2,56±0,78
	lys	8,02±1,15	6,59±1,30	8,75±2,00	7,05±200	7,13±1,32	6,02±1,12
	try	3,25±0,70	2,05±0,40	4,15±1,05	2,18±0,56	3,37±0,75	1,45±0,20
	his	9,56±1,09	7,18±0,65	10,32±1,56	8,39±1,2,8	9,92±1,12	6,24±1,07
	arg	10,35±2,05	8,27±1.20	11,50+2,25	9,13±1,20	10,42±1,00	7,75±1,18
	asp	18,54±2,62	15,78±2,14	19,12±2,56	16,21±2,52	18,66±2,35	13,60±1,20
Brain	ser	14,65±3,41	11,35±2,62	16,56±1,98	12,44±2,15	15,40±2,66	10,29±1,55
	gli	6,14±1,12	5,02±0,95	7,00±1,35	5,76±1,15	6,35±1,15	3,98±0,82
	glu	20,15±2,70	17,14±2,52	20,96±2,48	17,65±2,48	20,30±2,76	15,30±1,30
	βgla	11,44±1,41	9,56±1,38	11,56±1,45	10,02±1,30	11,32±1,56	8,26±1,42
	βala	5,37±1,97	4,92±1,56	5,48±2,00	5,00±1,95	5,35±1,75	4,05±1,10
	tyr	2,05±0,95	0,67±0,12	2,49±0,90	0,99±0,22	2,24±0,90	0,42±0,10
	met	5,18±1,24	4,00±0,79	5,88±1,07	4,36±0,87	5,35±1,30	3,30±0,76
	val	2,45±0,73	2,07±0,80	2,79±0,78	2,20±0,84	2,50±0,80	1,83±0,66
	phenala	1,83±0,57	0,75±0,10	1,80±0,60	0,80±0,16	1,74±0,60	0,45±0,15
	leu	3,56±0,96	3,20±0,75	3,97±0,63	3,40±0,78	3,65±0,87	2,90±0,75
Liver	cys	5,20±1,00	3,86±0,85	6,25±1,03	4,20±1,00	5,20±1,00	3,20±0,80
	lys	16,26±2,25	14,02±1,30	17,30±2,45	14,50±2,20	15,75±2,05	13,05±1,45
	try	5,18±1,27	3,20±0,80	5,26±1,20	3,62±1,00	5,37±1,30	2,88±0,36
	his	10,05+1,19	7,68±1,20	10,79±1,20	8,15±1,22	9,84±1,00	6,29±1,00
	arg	8,13±1,36	7,42±1,15	9,40±1,28	7,76±1,30	8,25±1,25	6,15±0,75
	asp	10,05±2,89	8,82±1,90	10,35±2,25	9,30±1,75	9,79±1,80	7,42±1,20
	ser	11,20±2,29	8,70±1,56	11,45±2,50	9,22±1,65	11,45±2,15	8,25±1,00
	gli	12,56±2,29	11,34±1,35	13,62±2,45	11,65±2,00	12,35±2,00	10,35±1,09
	glu	7,30±1,40	6,50±1,05	7,46±1,37	7,13±1,35	7,30±1,40	5,67±1,05
	βala	18,17±1,82	16,32±1,20	19,34±2,28	16,95±1,45	17,65±2,30	14,32±1,32
	prol	6,32±1,20	5,14±1,09	6,40±1,25	5,64±1,20	6,16±1,35	4,78±1,00
	tyr	3,54±1,00	1,06±0,32	3,85±1,16	1,55±0,40	3,25±1,15	0,80±0,20
	met	6,83±1,61	5,20±1,00	8,14±1,66	5,72±1,05	6,61±1,20	4,75±0,85
	val	7,74±1,34	7,21±1,15	7,95±1,30	7,34±1,15	7,28±1,42	6,20±1,00
	phenala	6,32±1,44	4,32±1,05	6,57±1,50	4,68±1,26	7,32±1,44	3,67±1,00
	leu	13,02±1,38	11,28±1,15	13,72±2,15	12,13±1,42	13,45±1,55	10,85±1,18

Proceeding from evidence reported in the literature and from our personal concepts on problems of chlorine intoxication, we undertook the task:
— to establish the changes in a number of amino acids and some enzymes under conditions of onefold and 5-day-long experimental poisoning with gaseous chlorine. More particularly, we were interested in the changes of blood, brain and liver amino acids: cysteine, lysine, tyrosine, histidine, arginine, aspartic acid (asparigine), serine, glycine, glutamic acid, ß-alanine, proline, tryptophan, valine, methionine, phenyl-alanine and leucine.
— to study the activity of cytochrome oxidase and succinic dehydrogenase in the same organic homogenates as a test, demonstrating the alterations of enzymes and their activity.

MATERIAL AND METHOD. The experiment was carried out on a series of 60 white rats, with mean weight 170 gr, divided in two basic groups according to nutritive diet. Each of the basic groups was further subdivided into three subgroups: control animals, poisoned a single time and poisoned during five days (Table 1).

The poisoning was performed in a gaseous chlorine chamber at 20 min exposure time. Bearing in mind the narrow zone between maximum allowable and minimal toxic concentrations, we applied a dose five times higher than the maximum allowable concentration. Our endeavour was to secure a longer lasting effect of chlorine upon the animals but we failed to achieve our aim, and the repeated poisoning lasted only five days, due to the poor general condition of the animals subjected to intoxication.

The activity of cytochrome oxidase was determined after the method of Vernon (10*) and of succinic dehydrogenase — after Kun and Abood (9). The changes in the free amino acids content were established according to Paschina (5).

Results and Discussion

The analysis of the results shows that in most of the amino acids investigated, *in both animal groups,* the *single-time poisoning* with chlorine, at 0,006 mg/*l* concentration, does not cause substantial changes (Table 2).

More significant are the changes in the blood, brain and liver cyclic amino acids-phenyl-alanine, tyrosine, tryptophan and histidine. The cysteine content is likewise substantially lowered by (25.86%). (Table 2).

The 5-day-long poisoning causes a stronger decrease in the concentration of the investigated amino acids (Table 2). Apart of these referred to above, the remaining amino acids investigated are also involved, with the exception of glycine, arginine, lysine and proline.

The onefold poisoning leads to insignificant inhibition of cytochrome oxidase in the studied organs, whereas the activity of succinic dehydrogenase remains unaffected (Table 3). This might be explained by the greater redox potential of the cytochrome oxidase.

In the animals, fed on *rich protein diet* (Group II), the values of the enzymes investigated, in the three organs, are slightly higher at the beginning of the experiment and after the poisoning (Table 3).

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* Modification suggested by the Research Laboratory on Nutrition—Higher Medical Academy, USSR.

Table 3

Enzymic Changes in Onefold Chlorine Poisoning

Enzyme	Homogenate	RATION			
		Controls	Poisoned	Controls	Poisoned
Cytohrome oxidase	Blood	18,10±1,40	14,00±1,63	20,50±2,08	16,20±1,60
	Brain	74,25±3,84	66,00±1,60	75,15±2,35	67,00±1,61
	Liver	60,25±1,37	54,75±2,50	61,17±1,50	55,50±1,54
Succinic dehydrogenase	Brain	176,50± 9,87	162,50±10,11	189,54± 7,56	182,00±2,82
	Liver	611,00±19,23	601,00± 8,35	688,20±15,30	675,70±9,18

Legend: Group I — rats fed on 18% cal protein
Group II — rats fed on 36% cal protein

The 5-day exposure of rats to the effect of gaseous chlorine at concentration 0.005 mg/l provokes greater changes in the activity of *cytochrome oxidase,* disclosing a 48,84% fall, the brain and liver ranking next. *Succinic dehydrogenase* is insignificantly inhibited, mainly affected is the cerebral (Table 4).

Table 4

Enzymic Changes in Repeated Chlorine Poisoning

Enzyme	Homogenate	Day of poisoning			Reduction %
		1	3	5	
Cytohrome oxidase	Blood	15,00±0,28	14,30±0,89	11,05±0,52	48,84
	Brain	66,11±1,28	59,05±2,12	46,38±1,56	38,67
	Liver	55,36±1,20	50,42±1,05	44,62±1,18	26,67
Succinic dehydrogenase	Brain	174,20±3,50	168,56±2,42	144,37±2,24	18,75
	Liver	610,00±5,10	600,20±1,18	587,64±2,00	3,60

The thorough analysis of the results obtained lays stress on several facts, which deserves a more detailed investigation and explanation. Mainly is concerned the involvement of the cyclic amino acids and cysteine, which might be due to their more pronounced activity. Attention is furthermore drawn to the stability of glycine, arginine and proline, which remain unaffected after the five-day chlorine exposure of animals. In all likelihood, this greater stability is related to their stronger resistance to oxidizing agents, in our case — chlorine.

Of no less interest is also the rather substantial reduction (18,75%) and greater affection of brain succinic dehydrogenase in the several-day exposures. The explanation of the latter fact might be linked to the cerebral asphyxia.

The conjectures set forward, concerning the changes in the amino acids referred to, assumed as being dependent upon their higher activity or resi-

stance, merely allude to a possible explanation of the inevitable phenomena, and are by no means in a position to afford a true explantion. The better understanding of the essence of these interrelationships is not object of the present study and will certainly require further experimentation.

Inference

The free blood amino acids cystein, phenyl-alanine, tyrosine, tryptophan and histidine were found to be the most labile to chlorine action. The content of glycine, arginine, histidine and proline remains unaltered in the five-day exposure to gaseous chlorine at 0,005 mg/l concentration.

The poisoning with small chlorine doses (exceeding 5 times the maximum allowable concentration) inhibits the acitivity of cytochrome oxidase. The strongest decrease following repeated poisoning is recorded in the activity of blood cytochrome oxidase, the brain and liver next ranking.

The activity of succinic dehydrogenase is lowered solely during repeated poisoning, with the brain activity being affected in higher degree.

The rich protein ration does not obviate the occurence of changes in the amino acids and enzymes studied in poisoning with low chlorine concentrations.

The early changes established in the free blood amino acids referred to above and in the activity of the blood cytochrome oxidase and succinic dehydrogenase require verification among chlorine contact workers, and only thereafter they could be given due consideration in the discussion of the chronic effect of chlorine.

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ВЛИЯНИЕ ЭКСПЕРИМЕНТАЛЬНОГО ОТРАВЛЕНИЯ ХЛОРОМ НА НЕКОТОРЫЕ АМИНОКИСЛОТЫ И ЭНЗИМЫ У КРЫС, ПОЛУЧАЮЩИХ БОГАТЫЙ БЕЛКАМИ РАЦИОН

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РЕЗЮМЕ

Сообщается об изменениях в содержании некоторых свободных аминокислот и об изменениях в активности цитохромоксидазы и сукциндегидрогеназы в крови, мозге и печени у белых крыс, находящихся на разных пищевых рационах, при отравлении хлором в концентрации 0.005 мг/л.

Отравление небольшими дозами хлора (в 5 раз выше предельно допустимой концентрации) ингибирует активность цитохромоксидазы. Сильнее всего снижается после нескольких кратного отравления активность цитохромоксидазы крови, следуемая мозга и печени.

Активность сукциндегидрогеназы понижается только при нескольких кратном отравлении, причем мозговая поражается в большей степени.

Богатый белками рацион не препятствует возникновению изменений в исследованных аминокислотах и энзимах при отравлении низкими концентрациями хлора.

Установленные ранние изменения в указанных свободных аминокислотах крови и в активности цитохромоксидазы и сукциндегидрогеназы крови требуют проверки среди контактных с хлором рабочих, после чего их можно иметь в виду при обсуждении хронического воздействия хлора.