CHANGES IN THE CONTENT OF CERTAIN AMINO ACIDS IN THE BLOOD AND LIVER OF WHITE ALBINO RATS, FED ON PROTEIN RATION DURING EXPERIMENTAL LEAD INTOXICATION

Communication II — histidine, arginine and valine

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The investigations carried out by Koelsch, Fr. (8), Gontzea I. and assoc. (7), Levy M. (9), Tzvetkov L. (5), Boyadjiev Vl. (1), Mitkova N., Kr. Kiriakov and P. Kolikovski (3) and others prove that the proteins prevailing in the alimentary ration of individuals working in contact with lead, accounts for a delayed occurrence and a more favourable course of lead intoxication. The explicitness of these inferences leaves unsolved the question about the effect of the specific proteins' composition, exerted upon the amino acid content in the blood and other organs during lead intoxication, and the posibility for regulating the changes ensuing by means of prophylactic diets, including proteins with an established qualitative content or various amino acids.

As a result of investigations on the issues stated above, our research team reached certain conclusions concerning the changes of cysteine, methionine and glutamic acid in the blood and liver of animals, fed on proteins and fatty rations during experimental lead poisoning (2). In the works referred to the regimen of feeding and intoxication of the experimental animals is described in detail, as well as the method for quantitative chromatographic determination of amino acids after T. S. Pashina, Z. S. Chulkina and M. I. Tumena etc (4). Poisoning is produced with lead acetate per os, whereas the protein content of the rations — regulated with casein. The animals of the first group are fed on rations poor in proteins, and the second — on high protein content ratiors.

Results

In the present work the results are reported of studying the changes in the concentration of the amino acids histidine, arginine and valine. Statistically elaborated after the method of analysis of dynamical changes and correlative relationships (Sepetliev, D.), they are summarized in table 1 and graphically illustrated in diagrams 1 through 6.

I. Changes in the blood.

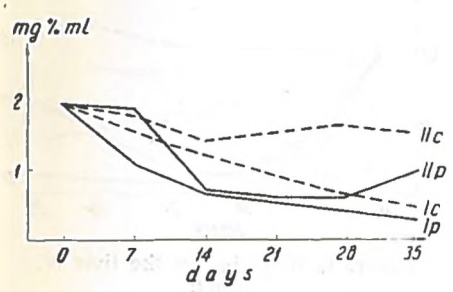
Lead intoxication causes reduction of the histidine in the blood of the poisoned and control animals of the first ration group (Diagr. 1, Table 1). The decrease has an acute onset, displayed as early as the first days of into-

Changes of Histidine, Arginine and Valine Content in the Blood and Liver of White Albino Rats, Fed on Protein Ration during Experimental Lead intoxication

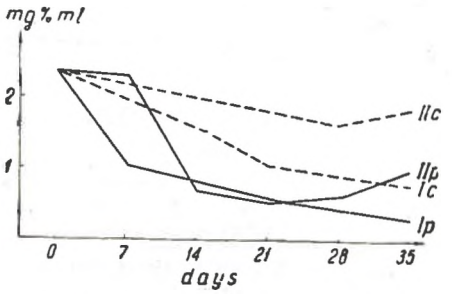
Amino acids	Substrate	Group	Ration	Prior to intoxication	At the end of the experiment	Average	σ^2 2	r	z	$t_{k/t}$ t_{tab}	P	$t_{I/II}$ t_{tab}	S/t
Histidine	Blood	poisoned (p)	I	1.9200	0.2620	0.5169	0.0379	0.929	1.6584	2.34>2.30	0,95		
		control (c)	I	1.9200	0.4300	0.7560	0.0808						
	mg/100 ml	poisoned (p)	II	1.9200	0.8971	1.3063	0.2267	0.999	3.8002	2.34>2,30	0.95	4.282>3.75	0.989
		control (c)	II	1.9200	1.7300	1.7410	0.0041						
	Liver	P	I	10.2300	1.0672	1.8690	0.0927	0.971	2.0923	4.431>3.36	0.99		
		C	I	10.2300	3.7590	5.5880	2.4456						
	mg/100	P	II	10.2300	6.5864	3.4164	5.8092	0.862	1.2933	9.445>5.04	0.99	3.995>3.92	0.999
r—	liver/blood	C	II	10.2300	11.0830	10.7090	0.230						
			I-0.95		II—0.98								
Arginine	Blood	P	I	2.4500	0.3390	0.6999	0.0561	0.91	1.5275	2.305>2.30	0.95		
		C	I	2.4500	0.8450	1.3604	0.2544						
	mg/100 ml	P	II	2.4500	0.9648	1.2459	1.0024	0.927	1.6584	2.33>2.30	0.95	2.601>2.30	0.95
		C	II	2.4500	1.8560	1.9250	0.0260						
	Liver	P	I	4.9000	1.0653	2.0712	2.6941	0.98	2.2976	2.301>2.30	0.95		
		C	I	4.9000	2.2120	3.0612	0.8860						
	mg/100 gr	P	II	4.9000	2.8543	2.5042	1.4580	0.997	3.2504	3.041>2.90	0.98	4.764>3.75	0.999
r—	liver/blood	C	II	4.9000	4.0927	4.5390	0.1531						
			I-0.99		II—0.994								
Valine	Blood	P	I	2.4500	0.0756	0.1799	0.0081	0.979	2.2976	5.733>5.04	0.999		
		C	I	2.4500	0.9260	1.4240	0.2038						
	mg/100 ml	P	II	2.4500	0.3063	0.7106	0.2965	0.996	3.1063	5.648>5.04	0.999	4.044>3.75	0.999
		C	II	2.4500	2.0240	2.2010	0.0144						
	Liver	P	I	8.4800	0.2920	2.9679	7.5426	0.84	1.2212	2.36>2.30	0.95		
		C	I	8.4800	2.0590	4.1854	4.611						
	mg/100 gr	P	II	8.4800	4.1602	4.7182	2.2094	0.98	2.2976	4.0120>3.92	0.99	5.382>3.92	0.999
r—	liver/blood	C	II	8.4800	8.3250	8.3130	0.0014						
			I-0.75		II—0.89								

xication. At the end of the first week, the concentration of histidine is almost 2.5 times lower than the initial one (1.9200 mg % — 0.7720 mg %ml). Next follows a stable, slow decrease in a rectilinear pattern and towards the 35th day from poisoning, the values are about 7.3 times lower than initially.

The decrease of histidine in the blood is likewise noted in the control animals fed on the same ration. The changes are about two times lesser than



Diagr. 1. Histidine content in the blood of rats:
Ration I and II. *C* — controls; *P* — poisoned



Diagr. 2. Arginine in the blood of rats
Ration I and II. *C* — controls; *P* — poisoned

in the poisoned. They might be explained merely with the disturbance of the amino acid metabolism, due to the paucity of proteins in the ration. The latter changes are considerably intensified by the effect of lead (Diagr. 1).

The rich in proteins ration produces insignificant changes in the histidine content in the blood of controls (Diagr. 1, Table 1). This favourable effect is reflected in the dynamics of the changes observed under the influence of the lead, the level of the latter being maintained constant up to the 7th day after poisoning. Thereafter, the concentration falls under the effect of progressively increased deposit of lead up to the 21st day. Subsequently an increasse is marked with a tendency towards normalization (Diagr. 1, Table 1). The final values of histidine in this group are twice as lower as the initial, whereas in the animals fed on poor in proteins rations, they are 7.3 times lower.

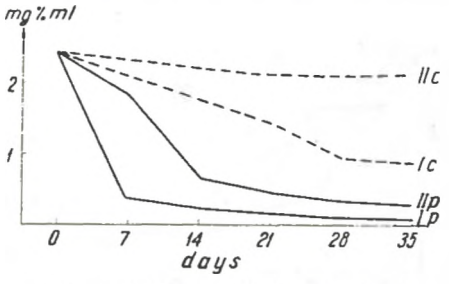
The arginine content displays deviations analogical to those of histidine. A decrease is established in the animals fed on a ration poor in proteins, which is marked in the poisoned animals.

The rich in proteins ration exerts an effect on the curve in the following fashion: the decrease noted up to the middle of the poisoning period is compensated and towards the completion of the experiment, the decrease of arginine in the animals fed on this particular ration is 2.5 times, and in those on poor in proteins ration 7.2 times higher as compared to the initial values. The changes noted in the controls fed on ration II are insignificant (Table 1, Diagr. 2).

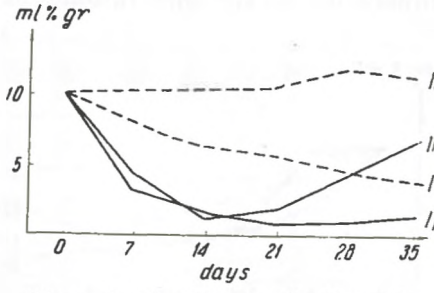
The content of valine follows the same patterns described for histidine and arginine. It is decreased after the exponential curve, steeper for the poisoned animals on ration I. The difference in the changes depending on the type of feeding is obvious. The rats on ration I exhibit four times lower con-

centration of valine in the blood compared to those on ration II followir a 35-day-long poisoning period. The reduction in the control animals c ration II is insignificant, a fact confirming the positive effect of the ratic (table 1, Diagr. 3).

Summarizing the results related to the changes of the bood amino aci investigated, the following basic inference could be drawn:



Diagr. 3. Valine in the blood of rats:
Ration I and II. *C* — controls; *P* — poisoned



Diagr. 4. Histidine in the liver of rats:
Ration I and II. *C* — control; *P* — poisoned

The concentratiors of amino acids — histidine, arginine and valine i the blood of the poisoned with lead acetate animals, are substantiall decreased, with those fed on poor in proteins ration displaying a stronge reduction.

II. Changes in the liver.

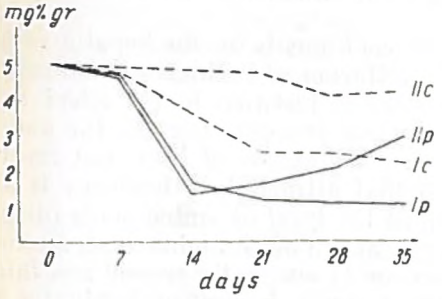
The histidine in the animals poisoned and fed on poor in proteins ration is much more reduced as compared to the reduction in animals on rich ra tion (Table 1, Diagr. 4). At completion of the experiment, its content is te times lower in the animals on ration I and 1.05 times in those on ration II Regardless of the initial decrease observed in animals fed on rich in protein ration, a clearly manifested tendency towards an increase and normalizatio is established after the 14th day. In the rats on poor protein ration, a con stantly low concentration — about 1.0147 mg % — is retained within period of 21 days after the poisoning. This rectilinear tendency for a decreas is likewise observed in the controls on ration I. The excess of proteins lead to a slight increase of histidine in the liver of the control animals, a fac related to the already described tendency towards normalization of its con centration in the poisoned animals.

The liver arginine is similarly influenced by alimentation (Table 1 Diagr. 5). In the control animals on poor protein ration it falls continuously. In those fed on a rich ration, a constant concentration is established following a minimal decrease.

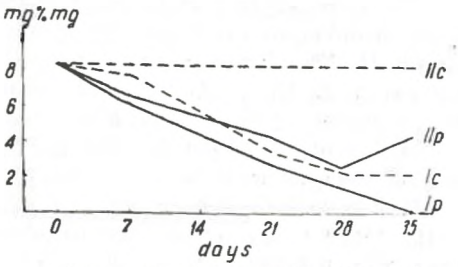
The effect of lead intensifies the changes in the hepatic arginine (Table 1, Diagr. 5). During the first week of the experiment the changes are insigni- ficant. About the 14th day a sharp decrease is observed in the animals of both groups. Subsequently, the arginine is decreased at a lower rate in the animals on ration I, and towards the completion of the experiment, the va- lues are about 4.5 times smaller than originally. The favourable effect of the rich protein diet as regards maintenance of the level of amino acids is

reflected by an increase of the arginine content after the 14th day, reaching towards the end of poisoning a level 1.04 fold lower than the initial, with a tendency to normalization.

The diet influences the content of hepatic valine. The control animals on rich protein ration retain a constant level with insignificant fluctuations around the average value 8.4800 mg %. In those fed on deficiency of proteins, the latter value is 4 times lower than the original.



Diagr. 5. Arginine in the liver of rats.
Ration I and II. *C* — controls; *P* — poisoned



Diagr. 6. Valine in the liver of rats.
Ration I and II. *C* — controls; *P* — poisoned

This background influences the changes in the concentrations of valine, brought about by the lead intoxication. As in the blood, the hepatic valine of the poisoned animals on ration I is decreased very acutely, in a steep rectilinear pattern with the final value — 0.2920 mg % — being about 30 times lower than that of the autocontrols — 8.4800 mg %.

The animals fed on rich protein diet do not show an acutely pronounced decrease up to the 28th day of poisoning. Thereupon, the valine content is raised and the final value results two times smaller than that in the autocontrol.

Discussion of the results

The summarized statistical analysis of the results plainly enough demonstrates that the changes established in the amino acids investigated are due to the exclusive effect of the lead, with a close correlative relationship existing between the value of alterations and the duration of poisoning. In support to this conclusion the values of t c p r, z, $t_{I/II}$ and p for the histidine, arginine and valine in the blood and liver of the poisoned and control animals are illustrated in table 1.

The decreased content of the listed amino acids in the blood and liver of the animals on ration I is assumed as a consequence to protein and some alimentary amino acids deficiency in the diet, accounting for disturbances in the protein and amino acid metabolism. The insiginificant decrease of the histidine and valine in the blood and of the arginine in the blood and liver of the control animals on rich protein ration, is very likely due to the same cause, as the casein, responsible for the regulation of the protein component of the experimental ration, introduces mainly methionine.

The comparative assessment of the data related to the concentration o histidine, arginine and valine in the blood and liver of the control and teste animals underlines the more favourable effect of the rich protein ratio (Table 1, coeff„z"t and p for the respective amino acids). It is evident fron the table that the statistical reliability for the arginine with p=0.01 i not sufficient. Hence, the protective effect of the rich protein ration shoul be verified on rather more numerous groups of animals, and only after du consideration has been given to the opinion stated herein concerning th effect exerted by larger doses of lead.

The comparative assessment of the „Z" coefficients for the hepatic amin acids doubtlessly confirms the favourable influence of the rich protein ratio (table 1). The drawing of a basic inference is justified to the effect tha the excess of the protein in the ration does not prevent affecting the amin acid balance in the blood and liver during the action of lead, but result in inhibiting its effect to such an extent that ultimately a tendency is ob served towards increase to normalization of the level of amino acids (diagr 1—6). The critical reductions in the concentrations of all amino acids studie in the blood and liver of the animals on ration II during the second and thir week of experiment, at a high level of the controls, is most probably due t the rather large lead dose applied during the first week of the experimen (200 mg/kg Pb^{2+}). It may be that the finding established is consequent t strong cumulative action. Accounting for the acute changes occurred, th dose for all the poisoned animals was subsequently reduced to 60 mg/kg Pb^{2+}, thus producing a more clear-cut demonstration of the effect exerted by the ration.

Nevertheless, the data obtained indicate that the lead very severely disturbs the amino acid spectre of the animals on both rations, an evident correlation being present between the changes in the liver and blood. In the rats on ration I, the respective coefficients are: $r_{hist}=0.95$, $r_{arg}=0.99$, $r_{val}=0.75$. With the rich protein ration, the coefficientris accordingly 0.98 for histidine, 0.994 for arginine and 0.89 for valine.

The very interesting results obtained could not explain the mechanism of the changes occurring in the amino acid metabolism during lead poisoning against the background of the respective alimentary regimen, as this was not the objective of our present investigation. It is necessary to carry out experiments on the regulation of the level of the amino acid spectre, through inclusion in the diet ration of proteins with a determined content or pure amino acids. The solution of these questions will greatly contribute to the elucidation of the actual problems of protective professional nourishment of a substantial group of workers, occupied in the lead production.

Inferences

1. The experimental lead poisoning causes a considerable impairment of the metabolism of the amino acids histidine, arginine and valine, manifested through a lowering of their content in the blood and liver of experimental animals. The greatest changes established are in the valine level.

2. The protein defficiency in the diet likewise leads to a reduced concentration of amino acids in the blood and liver, bringing about an intensification of the disorders in the poisoned animals.

3. The rich protein ration does not interfere with the occurrence of changes in the amino acids studied during poisoning with lead acetate, but substantially reduces the difference in their content between control and poisoned animals.

4. The results obtained pose for solution new problems, related to the elucidation of the mechanism of impairment of the amino acid metabolism during lead poisoning against the background of a determined dietary regimen. Further experimentation is required on the regulation of the disorders observed by inclusion in the diet of proteins with determined content or a complex of pure amino acids.

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ИЗМЕНЕНИЯ В СОДЕРЖАНИИ НЕКОТОРЫХ АМИНОКИСЛОТ В КРОВИ И ПЕЧЕНИ БЕЛЫХ КРЫС, НАХОДЯЩИХСЯ НА БЕЛКОВОМ РАЦИОНЕ, ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТРАВЛЕНИИ СВИНЦОМ

II сообщение — гистидин, аргинин, валин

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РЕЗЮМЕ

Токсическое действие свинца находится в связи с деструкцией клеточных и тканевых белков. Нарушения обмена белков можно успешно регулировать путем предохранительных пищевых рационов, составленных на основе точного знания изменений в аминокислотном составе крови и других органов, вызываемых отравлением. В этом аспекте были иссле-

дованы хроматографически по Т. С. Пасхиной количественные измене
аминокислот в крови и печени белых крыс, получающих пищу, бед
(3,70%) и богатую (34,67%) в отношении калории на счет жиров.

Экспериментальное отравление свинцом вызывает значительное
рушение обмена аминокислот гистидин, аргинин и валин, выражающе
в понижении их содержания в крови и печени опытных животных.
доимок белка в пищевом рационе также приводит к уменьшению их
центраций, усиливая нарушения у отравляемых животных. Богатый
ками рацион не останавливает возникновения изменений в исследован
аминокислотах при отравлении уксуснокислым свинцом, но значител
уменьшает разницу в содержании их между контрольными и отравляем
животными.