THE CONTENT OF CERTAIN AMINO ACIDS IN THE BLOOD ɪND LIVER OF WHITE ALBINO RATS, FED ON FATTY RATIONS DURING EXPERIMENTAL LEAD POISONING (HISTIDINE, ARGININE, VALINE)

Communication II

Vl. Boyadjiev, L. Halacheva, V. Nikolova

In the previous report on the problem discussed (2), the necessity was ;ubstantiated, accordingly backed with arguments, of investigating the amino acid changes in the blood and other organs of animals fed on fat ra- :ions, subsequent to experimental lead intoxication. The data available, 'eferred to the influence exerted by various amino acids upon the metabo- ism of lipids in the liver, other organs and biological surroundings under normal physiological conditions (6, 7, 8, 9, 10, 11), constitute the starting point of our studies. Some data relative to the effect of qualitatively diffe- rent fats upon the utilization of the proteins in various aminoacids in the organism were also employed (3, 5, 12). The literature survey did not give us sufficient information as to the kind and quantity of fats the daily ration of the threatened by lead poisoning should contain in order to prevent the occurrence of fatty dystrophy phenomena and the disturbance of protein me- tabolism. The quantitative and qualitative content of proteins is undo- ubtedly related to the earlier occurence and rather more severe course of saturnism. In the first report on the problem under discussion (2), a detailed description was given of the experimental background, regimen of nourishment and method of investigation of the animals and amino acids.

In the present paper the changes are described of histidine, arginine and valine occurring in the blood and liver of white rats, fed on poor fat ration (III) and rich fat ration (IV), subsequent to experimental lead poi- soning. The content of the listed amino acids in the blood and liver of the animals was determined through paper chromatography after the method of Pashina T. S. and Chulkina (4). The poisoning was produced with lead acetate per os. Quantitative regulation of the fats was accomplished with cod liver oil.

Results

I Changes of arginine, valine and histidine in the blood.

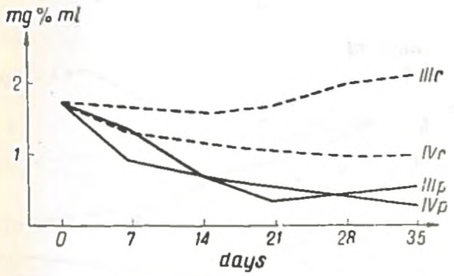
The statistical elaboration of the data obtained according to the methods of analysis of dynamic changes and correlative relationships led to the de-

Changes of Histidine, Arginine and Valine Content in the Blood and Liver of White albino Rats, Fed on Fatty Ration during Experimental Lead Intoxication

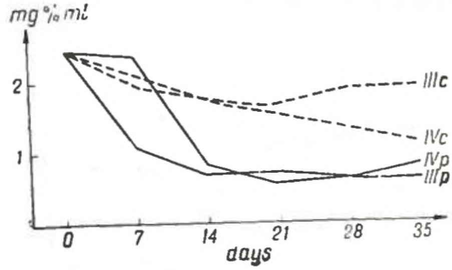
Amino acids	Substrate	Group	Ration	Average	σ²	r	Z	$t_{k/T}$ $t_{tag.}$	P	$t_{III/II}$ $T_{tag.}$	$S_{(t)}$
Histidine	blood	poisoned (p) control (c)	III III	1,0672 1,4590	0,8400 0,0694	0,76 —	0,9962 —	3,05 > 4,90 —	0,98 —	2,178 > 2,10	0,95
	mg/100 ml	p c	IV IV	0,5630 1,2410	0,0489 0,0437	0,89 —	1,4219	4,43 > 3,36	0,99		
	liver	p c	III III	2,2534 8,5520	0,7433 0,4120	0,93	1,6584	10,99 > 5,04	0,999	4,94 > 3,93	0,999
r—	mg/100 gr liver/blood	p c	IV IV	2,1324 11,6540	0,6545 2,6128	0,99	2,6466	10,92 > 5,04	0,999		
Arginine	blood	p c	III III	0,7752 1,8332	0,0572 0,0114	0,94	1,7380	7,818 > 5,04	0,999	1,81 > 2,10	0,95
	mg/100 ml	p c	IV IV	1,2800 1,5305	1,3022 0,1325	0,88	1,3758	2,309 > 2,30	0,95		
	liver	p c	III III	2,2750 4,7395	0,9695 0,0127	0,87	1,3331	4,727 > 3,36	0,99	0,69 < 2,10	0,95
r—	mg/100 gr liver/blood	p c	IV IV	2,9550 5,1542	0,7153 1,8772	0,90	1,4722	2,82 > 2,30	0,95		
Valine	blood	p c	III III	0,9790 1,9250	0,3834 0,0253	0,64	0,7928	2,833 > 2,30	0,95	5,196 > 3,92	0,999
	mg/100 gr	p c	IV IV	0,4156 1,1710	0,0416 0,0749	0,95	1,8318	4,529 > 3,36	0,99		
	liver	p c	III III	6,7855 7,0567	2,4782 0,1693	0,98	2,2976	2,489 > 2,30	0,95	0,00 < 1,73	0,90
r—	mg/100 gr liver/blood	p c	IV IV III	1,8580 4,3155	0,3169 1,4629	0,98	2,2976	3,824 > 3,36	0,99		

termination of certain regularities in the changes of the content of the amino acids investigated (Tabl. 1, Diagr. 1—6).

The histidine, arginine and valine concentrations in the blood of all the animals of both ration-groups are lower in comparison to those of the auto-



Diagr. 1. Histidine content in the blood of rats.
Ration III and IV. *C* — controls; *P* — poisoned



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

controls. The changes are substantially more seldom demonstrated in those poisoned with lead acetate.

The effect of the alimentary rations is more clearly outlined in the control animals. The concentrations of the three amino acids are lower in the rats on ration IV.

A constant lower level of histidine and valine is established (Diagr. 1, 3) (1.0700 mg % for the histidine and 0.915 mg % for valine) not earlier than the 38th day (28th day according to the diagrams), i. e. following a protracted alimentary adaptational period.

The arginine is reduced in a rectilinear pattern throughout the entire term of investigation (Diagram 2).

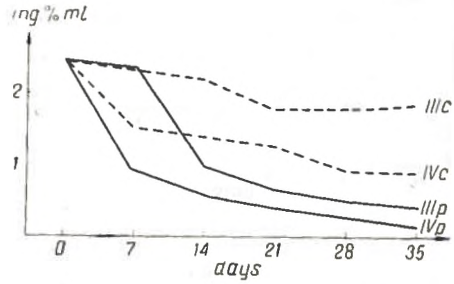
The animals fed on deficient fat diet display a decrease of histidine, manifested in a slightly convex curve with a minimum value towards the 24th day and normalization about the 38th day since commencement of feeding (28 according to the diagram). A similar tendency in the changes is established with the arginine as well, the latter values being lower than those in the autocontrols. The valine concentration in the control animals on ration III is insignificantly reduced and beginning with the 31st day (corresponding to the 21st day of the diagram) it is maintained constant.

The poisoned animals, fed on rich of fats rations reveal inferior data relative to histidine and valine (Diagr. 1 and Diagr. 3). Towards the end of the experiment the valine shows a 13.5 fold reduction in the animals on ration IV against a 6-fold reduction in those on ration III. The decrease of the histidine is accordingly 6-fold in the rich and 3.3 fold in the poor-fat-ration group. The difference for the arginine found among the poisoned animals of the respective groups is not reliable.

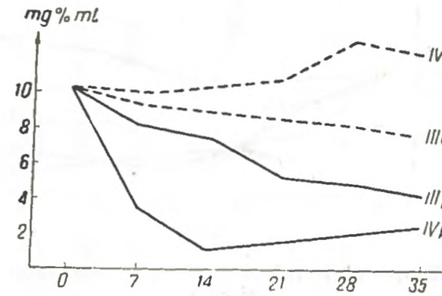
II Changes of the amino acids histidine, arginine and valine in the liver.

The changes in the amino acids studied, observed in the liver of the test animals are identical to those found in the blood, but rather more significantly influenced by the lead poisoning and alimentary rations.

The liver histidine decreases insignificantly in the control animals on ration III, whereas those fed on excess of fats (ration IV), display, subsequent to a gradual increase with a maximum on the 38th day since nourishment (28th in the diagram), a reduction with a tendency towards normalization (diagram 4).



Diagr. 3. Valine content in the blood of rats.
Ration III and IV. *C* — controls; *P* — poisoned



Diagr. 4. Histidine in the liver of rats.
Ration III and IV. *C* — controls; *P* — poisoned

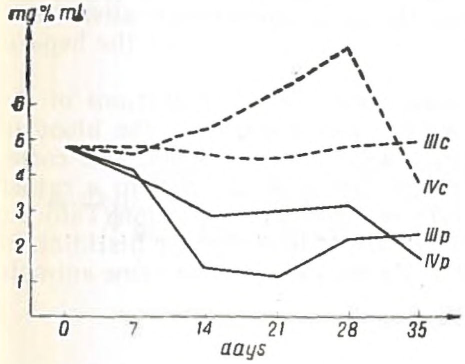
Lead poisoning intensifies the changes observed in the histidine content (Diagr. 4). Among the animals on ration III, its concentration gradually decreases in a step-like curve pattern, disclosing values two times lower than the initial ones. Among the rats on ration IV, the reduction runs a steep exponential curve up to the 14th day, and thereafter begins a slight increase till the end of the experiment (diagr. 4), the final value being four times lower than the initial.

The changes in hepatic arginine display differences as compared to the regularities established with the other amino acids. More frequent fluctuations are noted in the controls, as well as in the poisoned animals of both rations. The arginine concentration in the control animals on a rich fat diet is altered in a wavy pattern with a minimum on the 17th and 45th day and a peak on the 38th day (7th, 35th and 28th according to diagram 5). In those fed on deficient fat diet, the level is constant.

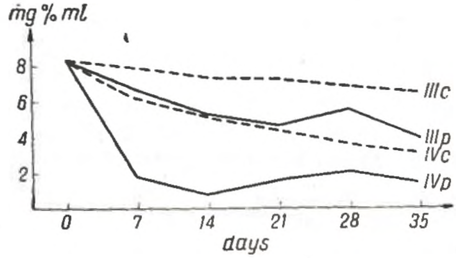
The content of hepatic arginine in the animals poisoned of both groups is insignificantly reduced up to the 7th day since intoxication (Diagr. 5). In the course of the second week of experimentation certain differences are marked. The arginine among the rats on ration III falls abruptly up to the 14th day, is retained constant up to the 21st day and is gradually increased thereafter until completion of the experiment. Among the ration IV animals the reduction tendency is rather more constant with maintaing of an unaltered level for a duration of two weeks, corresponding to the increase in the controls and a new reduction up to the 35th day.

The valine in the lever of the control and poisoned animals of both groups falls. In the controls the changes are insignificant for the rats on ration III and rather pronounced in those on ration IV (Diagr. 6).

The poisoned animals of both groups display symmetrical changes. A gradual decrease for those of the III group, and a steep one — for the control of the IV group. After the 21st day a tendency is established towards raising with a peak value on the 28th day and a new slight decrease till the end of the experiment. The latter values are about 6 times lower as compared to the initial concentrations of the valine in the autocontrols of group IV and 1.6 times for those on ration III.



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



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

Discussion of Results

The analysis of the results shows that the fat rations account for essential changes in the quantity of blood and hepatic amino acids — histidine, arginine and valine. The changes reported are rather insignificant in the animals fed on poor fat diet. They are due most probably to a deficiency of the respective amino acid in the content of the food or to a slower alimentary adaptation. The data for arginine in the blood and liver (Diagrs. 2 and 5) warrant the inference just drawn.

The changes in the amino acids studied are much greater in the animals on alimentary ration IV, and particularly pronounced insofar hepatic amino acids are concerned. They might be explained with the impairment of the protein, respectively amino acid metabolism, fatty dystrophy of the liver and other organs, usually occurring in alimentary fat excess. A great number of literature reports exist on this particular issue.

The concentrations of the studied amino acids undergo more significant changes under the effect of lead, very frequently exhibiting a several-fold reduction beneath their level in autocontrols. The extent of changes is in close relationship with the quantity of lead received, the duration of the experiment and the degree of lead poisoning (Tabl. 1 for $t_{c/p}$).

The coefficients computed for the histidine, arginine and valine in the blood of the animals on both rations support the inference just reached (tabl. 1 $t_{III/IV}$ for individual amino acids with corresponding p). In all instances S(t) is greater than 0.95. Analogical is the statistical reliability for the dependence of the changes in hepatic amino acids on the degree of poisoning (Tabl. 1 for t_{III} and t_{IV} with the respective p in hepatic amino acids).

The Z-coefficients were compared with a view to obtaining statistical confirmation of the differential importance of the ration and poisoning, resulting in a strong and close dependence between the changes in the amino acids and the degree of lead poisoning (Tabl. 1 for r, z, $t_{III/IV}$ and p). From the data reported it is evident that arginine in the blood and liver appears to be influenced merely by lead poisoning. The quantitative content of the fatty ration (poor or rich) does not preclude the occurrence of these alterations, i. e. it exerts no protective effect whatsoever. The inferences for the hepatic valine are identical.

In the course of studying the influence upon the concentrations of the hepatic amino acids investigated, exerted by their content in the blood of the poisoned animals, monotype regularity was not established. The correlative coefficients computed for the various amino acids vary in a rather wide range between close, great, significant and moderate correlation (Tabl. 1 r liver) blood for the various amino acids). If the dependence for histidine in animals on ration IV is very strong r = 0.92, for the valine in the same animals it is moderate r = 0.48.

Inferences

1. The fat rations studied, regardless of their quantitative content, produce a reduction of amino acids (histidine, arginine and valine) in the blood of the control animals. Merely the valine is lowered among the hepatic amino acids, whereas the histidine and arginine in animals fed on poor fat ration are considerably decreased; in rich ration they reveal an increase with a peak on the 38th day since alimentation and a new decrease till the end of the experiment.
2. Lead poisoning accounts for several fold increase of differences between the initial and final values of amino acids in the blood and liver of the rats of both ration groups.
3. The rich in fats ration exerts a more unfavourable effect on the content of histidine and valine. The effect established of the poor fat ration preventing the reduction of the amino acids appears to be unsatisfactory, but anyway, in favour of the concept recommending the securing of poor in fats alimentary diet for individuals threatened by lead poisoning.

REFERENCES

1. Бояджиев, Вл. Влияние на някои белтъчни и мастни режими за възникване и протичане на оловното отравяне. — *Научни трудове на ВМИ — София*, 1962, т. 41, в. 5, 83—98.
2. Бояджиев, Вл., Л. Халачева и П. Николова. Съдържание на някои аминокиселини в кръвта и черния дроб на бели плъхове, хранени с мастни дажби при хронично оловно отравяне — I съобщение. — Резюмета на втората национална конференция по хранене, София, окт. 1965, с. 38.
3. Горшкова, Л. С. и А. А. Дородница. Влияние различных жиров на питательную ценност белка. — Мед. лит. СССР, 1935, с. 137.
4. Пасхина, Т. С. Современные методы биохимии. М., 1964, 29, под ред. Ореховича.
5. Пикат, А. К. и О. Я. Курцины. О влиянии качественно различных жиров в пищ на обмен азота. — *Вопр. пит.*, т. XII, 6. с. 34—37, 1953.
6. Кеупап, А., U. Strecker. — *J. Biol. Chem.*, 211, 883, 1954.

7. Lerner, E. M., J. H. Muller. — *J. Biol. Chem.*, 181, 43, 1949.
8. Mclwain, H., J. A. Hoper, D. Hughes. — *Biochem. J.*, 42, 492, 1948.
9. Vigneand, V., J. P. Chandler, A. W. Mayer, D. M. Keppel. — *J. Biol. Chem*, 31, 57, 1939.
0. du Vigneand, V. — *Biol. Symposia*, 5, 234, 1941.
1. Williams, J. N. and A. J. Hurlebans. — *Jour. of Nutrition*, 85, 1, 73, 1905.
2. Shay, H., R. Kolm, S., S. Fels. Changes in renal wather output of albino rat ander influence of high diet. — *Gastroenterology*, 4, 257—265, 1945.

СОДЕРЖАНИЕ НЕКОТОРЫХ АМИНОКИСЛОТ В КРОВИ И ПЕЧЕНИ БЕЛЫХ КРЫС, НАХОДЯЩИХСЯ НА ЖИРОВОМ РАЦИОНЕ, ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ОТРАВЛЕНИИ СВИНЦОМ

II сообщение — гистидин, аргинин, валин

Вл. Бояджиев, Л. Халачева, В. Николова

РЕЗЮМЕ

Знание изменений аминокислотного состава крови и печени является верной основой для составления пищевых рационов, предотвращающих возникновение сатурнизма. В данной работе сообщаются результаты, касающиеся изменений аминокислот: гистидин, аргинин и валин в крови и печени у отравляемых уксуснокислым свинцом белых крыс, получающих пищу, бедную (4,09%) и богатую (33,86%) в отношении калорий на счет жиров. Количественное определение аминокислот производилось хроматографически по Т. С. Пасхиной.

Исследованные богатые жиром рационы, независимо от их количественных составов, вызывают снижение вышеупомянутых аминокислот в крови контрольных животных. Снижается количество и печеночного валина. Гистидин и аргинин в печени, получающих бедный жирами рацион, понижаются значительно, а при богатом — первоначально повышаются, а затем резко падают.

Отравление свинцом увеличивает несколькократно разницы между начальными и конечными значениями аминокислот. Богатый жирами рацион оказывает более неблагоприятный эффект в отношении содержания гистидина и валина. Установленный предохраняющий от снижения количества аминокислот эффект бедного жирами рациона является неудовлетворительным, но все же является в пользу воззрения, что застрашенные отравлением свинцом следует чтобы получали бедный жирами пищевой рацион.