CHANGES IN THE CONTENT OF SOME AMINO ACIDS IN THE BLOOD AND URINE FOLLOWING EXPOSURE TO LOW TEMPERATURE AND IONIZING RADIATION

Yu. K. Ledentsov and E. M. Larionova

Department of Biochemistry (Director, Dotsent A. M. Genkin),
Sverdlovsk Medical Institute
(Presented by Member AMN SSSR A. V. Lebedinskii)
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It has been shown by numerous workers that ionizing radiation can, at certain dosage levels, cause substantial changes in various aspects of metabolism. These included regular shifts in nitrogen metabolism, expressed by a negative nitrogen balance and increases in the contents of a number of nitrogenous components of the urine – uric acid, urea, purine bases, ammonia, and amino acids [3, 6, 8, 10]. Aminociduria has been reported in cases of human radiation sickness [9].

Only a few papers have been published dealing with the metabolic effects of radiation sickness complicated with other factors. It was found that exposure of albino rats to ionizing radiation or to low temperatures, or to these two factors simultaneously, was followed by a fall in total plasma protein content, to changes in the relative proportions of its protein fractions, and to a fall in the rate of incorporation of labelled amino acids into proteins [1].

Investigation of the disturbances in nitrogen metabolism following exposure to the above factors may facilitate systematic studies of the changes in metabolism of the individual amino acids. The object of the present research was to make a comparative study of the contents of free amino acids of the blood and urine of animals exposed to ionizing radiation, localized cooling, and to a combination of these two factors.

EXPERIMENTAL METHODS

We used 40 male white rats, of body weight 120-160 g, in our experiments. The animals were divided into four groups: a control group, a group exposed to localized cooling, an irradiated group, and a group exposed to low temperature after irradiation.

The rats were irradiated from an RUM-3 source, filtered through 0.5 mm of Cu and 1 mm of Al, current strength 15 ma, voltage 190 kw, skin-focus distance 40 cm, total dose 700 r.

The rats were cooled by placing them in an open glass container, the bottom of which was immersed in freezing mixture (CO_2-snow, ice, salt) at $-40°$ for 4 h. The body temperature of the rats was measured by means of an electro-

TABLE 1. Changes in the Contents of Free Amino Acids in the Blood of Rats at Different Times after Irradiation (Mean Results of 10 Experiments)

Amino acid	Control		Days after irradiation					
			3rd day			7th day		
	mg-%							
Glutamic acid	1,49	0,23	0,89	0,15	0,05	3,19	0,16	0,001
Leucine	1,54	0,9	0,81	0,13	0,1	2,97	0,46	0,02
Alanine	0,94	0,32	0,84	0,57	0,2	1,72	0,12	0,001
Aspartic acid	0,80	0,13	0,42	0,21	0,05	2,05	0,37	0,02
Glycine + serine	1,57	0,67	1,24	0,18	0,1	3,47	0,27	0,001
Cysteine	0,53	0,23	0,52	0,26	0,5	3,3	0,21	0,001
Methionine*	1,03	0,25	0,92	0,55	0,5	—	—	—
Tyrosine	1,41	0,10	1,62	—	0,5	2,62	0,41	0,001

*Not detected in all the experiments

TABLE 2. Content of Free Amino Acids in the 24-Hour Collection of Urine of Irradiated Rats at Different Times after Exposure (Mean Results of 10 Experiments)

Amino acid	Control		Days after irradiation					
			3rd day			7th day		
	mg							
Glutamic acid . .	111	12	52	6,0	0,001	111	20	—
Leucine	98	27	102	25	0,2	102	51	0,5
Alanine	82	10	64	10	0,2	241	82	0,05
Aspartic acid . .	66	12	31	6	0,02	17	10	0,01
Glycine + serine	210	30	78	10	0,001	165	50	0,2
Cysteine.	39	14	20	—	0,2	21	16	0,2
Methionine . . .	31	12	21	10	0,5	51	41	0,5
Tyrosine.	53	20	45	13	0,5	31	20	0,2

thermometer in the region of the neck (it did not fall below 36°); the temperature of the muscles of the cooled extremities fell to 15-20°. The effect of cooling was to cause second to third degree frostbite of the hind legs. The skin became hyperemic, and later on cyanotic, with pronounced edema extending to the subcutaneous connective tissue layer, with small patches of darkening.

The rats were placed in a metabolism cage, for collection of their daily urine output. The samples collected before irradiation, and on the 3rd and 7th days following irradiation, were taken for analysis. Blood samples were taken at the same times.

The rats which had been exposed to low temperature were killed by decapitation 24 h after exposure. The free amino acid contents of their blood and urine were determined by the method of one-way ascending paper chromatography. The irradiated animals were taken for analysis during the latent period of radiation sickness, and at the height of its development. In the latter case, the animals displayed pronounced leucopenia (1200-1500 leucocytes in 1 mm^3 of blood), adynamia, anorexia, fluid motions, hemorrhages on the lips and ears, and loss of body weight.

In the last series, the rats were exposed to low temperature on the 7th day after irradiation. Frost-bite developed differently in these animals: hyperemia of the skin where it had been in contact with the cold surface was less pronounced, the brostbitten extremities were markedly cyanotic, the blisters occupied a greater part of the skin surface, and frequently contained bloodstained fluid, the tendency towards necrosis was greater, and healing proceeded more slowly.

Chromatography of amino acids was done by the method of Bode and Giri, as modified by Zaitseva and Tyuleneva [2, 5, 7], and blood and urine were analyzed for cysteine, glycine, serine, glutamic acid, aspartic acid, alanine, leucine, tyrosine, and methionine. The results were subjected to statistical treatment.

EXPERIMENTAL RESULTS

The data of Table 1 show that there was an appreciable fall in the glutamic and aspartic acid contents of the blood by the 3rd day after irradiation, while on the 7th day there was a statistically significant rise in the contents of all the amino acids examined.

There was a substantial fall in the amounts of glutamic and aspartic acids, and of glycine + serine excreted on the 3rd day after irradiation (Table 2).

There was also a tendency towards diminished excretion of the other amino acids, the 24 h output being lower on the 3rd day than in the control group. On the 7th day there was a considerable rise in excretion of alanine, but a further fall in excretion of aspartic acid. Excretion of the remaining amino acids fell within the normal physiological range.

Thus, there was a fall in the contents of free amino acids in the blood and urine during the initial period of radiation sickness (3 days). This effect appears to be attributable to the condition of negative nitrogen balance associated with development over this period of partial protein deprivation.

At the height of radiation sickness (7th day) the content of all of the amino acids rose in the blood, which may have been due to processes of breakdown of proteins prevailing over their synthesis in most of the organs. Breakdown of tissue proteins proceeds with particular intensity when the condition is complicated by other morbid processes [4]. Pronounced aminoaciduria was not, however, encountered at this stage. With the exception of alanine, the output of which showed an increase, the amounts of other amino acids excreted in the 24 h period did not differ significantly from those encountered in the control group, and the aspartic acid content was even lower.

The contents of amino acids in the blood of rats subjected to localized cooling following irradiation showed an increase, but this effect, recorded 24 h after cooling, was not statistically significant. It is conceivable that the excess of amino acids had been excreted by the time of sampling.

TABLE 3. Changes in the Contents of Free Amino Acids in the Blood of Rats Exposed to Low Temperature Following Irradiation (Mean Results of 10 Experiments)

Amino acid	Control		Frostbite			Irradiation (7th day)			Irradiation + frostbite		
	mg-%										
	M	m±	M	m±	P	M	m±	P	M	m±	P
Glutamic acid	1,49	0,23	1,66	0,32	0,5	3,19	0,19	0,001	2,59	0,44	0,05
Leucine	1,54	0,95	2,43	0,84	0,2	2,97	1,12	0,02	4,21	0,54	0,05
Alanine	0,94	0,32	1,21	0,65	0,1	1,72	0,44	0,001	1,76	0,29	0,01
Aspartic acid	0,80	0,13	1,34	0,32	0,1	2,05	0,34	0,02	1,33	0,30	0,1
Glycine + serine	1,57	0,67	2,30	0,51	0,1	3,47	0,27	0,001	2,79	0,67	0,1
Cysteine	0,53	0,23	0,73	0,31	0,5	3,30	0,21	0,001	1,38	0,37	0,05
Methionine	1,03	0,25	0,74	0,13	0,2	—	—	—	2,17	0,55	0,05
Tyrosine	1,41	0,10	1,63	0,50	0,5	2,62	0,41	0,001	2,09	0,48	0,2

TABLE 4. Changes in the Contents of Free Amino Acids in a 24-Hour Output of Urine of Rats Exposed to Low Temperature Following Irradiation (Mean Values)

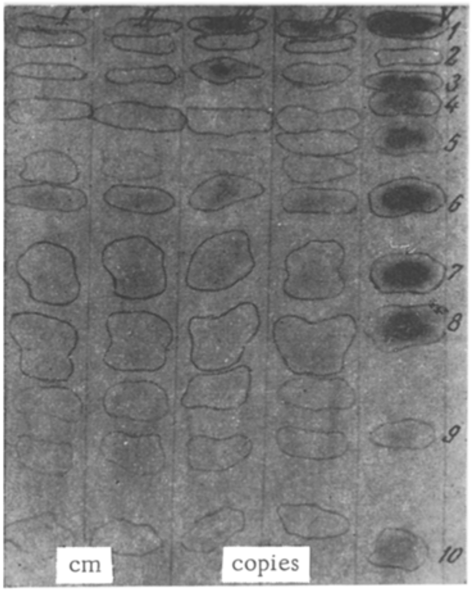
Amino acid	Control		Frostbite			Irradiation (7th day)			Irradiation + frostbite		
	mg-%										
	M	m±	M	m±	P	M	m±	P	M	m±	P
Glutamic acid	111	12	308	60	0,001	111	20	—	80	10	0,1
Leucine	98	27	51	16	0,2	102	51	0,5	36	8,6	0,1
Alanine	82	10	678	161	0,01	241	84	0,1	249	88	0,1
Aspartic acid	66	12	147	53	0,1	17	10	0,01	61	24	0,5
Glycine + serine	210	30	312	44	0,05	165	50	0,2	128	40	0,1
Cysteine	39	14	128	32	0,05	21	16	0,2	37	15	0,5
Methionine	31	12	45	15	0,5	51	41	0,5	43	16	0,5
Tyrosine	53	20	136	36	0,05	31	20	0,2	56	10	0,5

The urinary output of amino acids rose 24 hours after exposure to cold. Statistically significant increases were registered for glutamic acid, glycine + serine, cysteine, tyrosine, and particularly for alanine, the output of which was 8 times higher than in the control group. There was little change in excretion of aspartic acid, and a fall in excretion of leucine.

The rise in free amino acid content of the blood following exposure to low temperature may thus be regarded as constituting part of a compensatory reaction of the organism, directed towards mobilization of the energy reserves for maintenance of thermoregulatory processes, while increased urinary excretion would be a consequence of the raised blood levels.

When the action of low temperature was added to that of radiation (Tables 3 and 4) the contents of all of the amino acids rose in the blood. This increase was statistically signficant for glutamic acid, leucine, alanine, cysteine, and methionine (Fig. 1). This effect was not, however, accompanied by any considerable rise in urinary excretion of amino acids, as had been found to occur in frostbitten animals. Except for alanine, urinary excretion of amino acids was the same in this as in the control group. Alanine excretion was raised 3-fold (Fig. 2), while leucine excretion fell.

Our findings show that the amino acid metabolism of rats suffering from both radiation sickness and frostbite differs appreciably from that of rats suffering from frostbite alone.



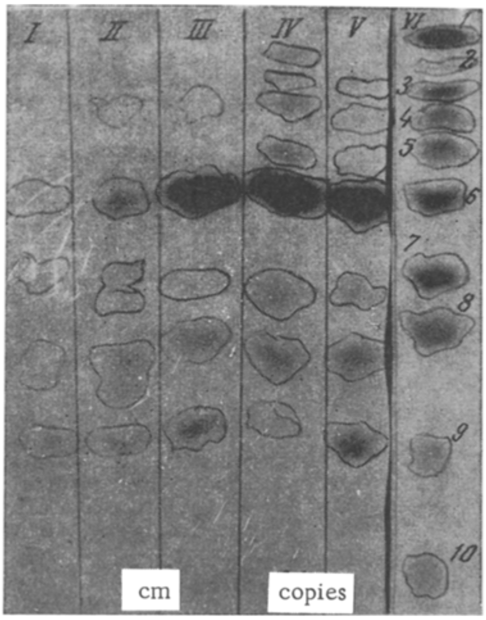


Fig. 1. Fig. 2.

Fig. 1. Chromatograms of rat blood. I) Control group; II) frostbitten rats; III) frostbite on the 7th day after irradiation IV) the same; V) reference spots: 1) leucine, 2) phenylalanine, 3) methionine, 4) tryptophan, 5) tyrosine, 6) alanine, 7) glutamic acid, 8) serine + glycine, 9) aspartic acid, 10) cysteine.

Fig. 2. Chromatograms of rat urine. (I) Control group; II) 3rd day after irradiation; III) 7th day after irradiation; IV) frostbitten; V) frostbitten on 7th day after irradiation; VI) reference spots. Other designations as in Fig. 1.

SUMMARY

The free amino acid contents of the blood and urine of rats suffering from radiation sickness, from frostbite, or from a combination of these conditions have been determined.

The values found varied at different stages of radiation sickness, at the height of which high levels of all the amino acids were found in the blood, without concomitant aminoaciduria, except for alanine. Urinary excretion of amino acids was raised significantly in frostbitten animals.

Blood amino acid levels were all raised in rats suffering from both radiation sickness and frostbite. Urinary excretion of alanine was raised, and of leucine lowered; the output of the other amino acids did not differ from that of the control group.

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