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CHANGES IN PROTEIN METABOLISM OF ALBINO RATS AT INDUCED AMMONIA STRESS

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ABSTRACT

The present study aims at understanding the alterations in the protein metabolism of albino rats at induced ammonia stress. Albino rats were induced to sub lethal dose of 1.16 ppm of ammonium sulphate until delivery. The total proteins, free amino acids, urea, ammonia and arginase activity were estimated in tissues of brain, liver and kidney in male, female and litters. The changes in these parameters were discussed.

KEYWORDS: **Ammonium sulphate, Biochemical parameters, Albino rats.**

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INTRODUCTION

Fertilizers have a significant role in the every day life of modern cultivation. Use of the super chemicals has become indispensable for fighting with the production of crops and entering into the food chain of different animals. However, indiscriminate use of the chemicals has resulted in imposing a serious threat to human health as they leave residues in the food and produce ill effects, when the concentration exceeds safe tolerance level.Proteins are dynamic molecules whose functions almost invariably depend on interactions with the other molecules and these interactions are affected in physiologically important ways by sometimes subtle, some times sticking changes in proteins confirmation. An important goal of molecular medium is the identification of proteins, whose presence, absence or deficiency is associated with specific physiological state or diseases[1]. Protein on the other hand is primarily used for non fuel purposes but can under certain circumstances, serve as an energy source[2]. Hydrolysis of dietary protein and endogenous protein which result in the formation of amino acids maintain homoeostasis in protein environment because amino acids are under constant movement between tissue and plasma. The physiological state of the cell is dependent upon its free amino acid pool[3]. Ammonia is found exogenously by the microbial action on dietary protein and urea present in the fluids. Hyperammonemia in the body causes flapping tremors, blurring visions and severe cases like coma and death[1].Urea production has an additional function in the maintenance of osmotic pressure and in the production of arginine and ornithine. Conversion of ammonia to urea is one of the important mechanisms of ammonia detoxification in fresh water fishes[4]. Arginase is concerned with ammonia conversion. It is a metal protein which is responsible for the splitting of amino acid, arginine; catalyses the hydrolytic conversion of arginine to ornithine and urea. Use of fertilizers of some ammonia products is a matter of great concern of human and animal health. Therefore, study has been undertaken to assess the impact of ammonia on albino rats. For this purpose, biochemical investigations have been undertaken to determine the effect of toxic ammonia on brain, liver and kidney of both sex and litters of Wistar albino rats, the results of which have been reported in this paper.

MATERIALS AND METHODS

The LD_{50} dose of ammonium sulphate to albino rats over 48 hours was reported as 4.82 ppm, and thereby 1.61 ppm dose as sub lethal[5]. The male and female albino rats of same age groups were separated into 6 sets. Each set was maintained in separate cages. Out of six sets, three sets were considered as experimental and remaining as control animals. After 10 days, male and female rats of 6 sets were separated and the experimental sets were treated with sub lethal dose until delivery. The adult rats and litters were sacrificed after 72 hours of delivery by cervical dislocation and then different metabolically functional tissues such as liver, brain and kidney were isolated for analysis. The total protein content in the tissues of control and experimental samples was estimated by the method of Bradford[6]. Free amino acids were estimated by the method of Colowick and Kalpan[7]. Urea content was estimated by the diacetyl monoxide method as described by Natelson[8]. Ammonia was estimated by the method of Sadasivudu et al.[9] Arginase activity was estimated by the method of Beruter et al.[10]Institutional Animal Ethics Committee, Sri Venkateswara University, Tirupati, India (Regd.No.438/01/a/CPCSEA, dated 17-7-2001) resolved in Resolution No.02/2006-2007(i)/a/CPCSEA/IAEC/SVU/PN-AS dated 14-09-2007 to permit the author to take up a research work entitled “Certain metabolic changes in the selected tissues of albino rat under ammonia stress”. The ethical committee examined the importance of the small animal use in the present investigation and it has no objection for the usage of the Albino rat as per the guidelines of the Institutional Animal Ethics Committee, S.V. University, Tirupati, India.

RESULTS AND DISCUSSION

The data given in tables from 1 to 5 shows the changes in total proteins, free amino acids, urea, ammonia and arginase activity levels in brain, liver and kidney tissues of control and ammonia exposed albino rats and litters. Ammonia caused the change in all the above parameters and the changes were found to be statistically significant over the control. The increase of total proteins and urea in the brain and the decrease in the liver and kidney of adult rats and litters shown in Tables 1 and 2 suggests that the decreased protein content might be attributed to the destruction or necrosis of cellular fraction and consequent impairment in protein synthesis machinery[11]. The decrement in protein level can be correlated to the increase of free amino acid level observed in the liver and kidney and decrease in brain tissue. Being the most important organic constituents of organs, the role of proteins in the compensatory mechanism of animal can be accepted during stress conditions[12]. Shift in proteins may ultimately lead to alterations in the entire protein metabolism of animals.

Table 1

EFFECT OF AMMONIUM SULPHATE ON TOTAL PROTEIN CONTENT (µ moles of albumin/g wet wt. of tissues) IN THE TISSUES OF BRAIN, LIVER AND KIDNEY OF MALE AND FEMALE RATS AND LITTERS

Tissues	Adults				Litters	
	Males		Females		Control	Experimental
	Control	Experimental	Control	Experimental		
Brain M	68.42	76.32	64.24	74.34	59.64	68.25
SD	± 4.14	±5.32	±3.98	±5.46	±3.25	±3.72
PC		+11.54		+15.72		±14.43
Liver M	136.24	116.42	138.28	119.24	132.36	119.64
SD	±6.46	±6.26	±5.63	±6.72	±6.34	±5.94
PC		-14.54		-13.76		-9.61
Kidney M	118.42	104.36	120.62	108.32	117.36	96.47
SD	±5.43	±5.72	±6.46	±5.72	±6.35	±6.21
PC		-11.87		-10.19		-17.79

All the values are mean ± SD of 6 individual observations.
PC = Percent change over control
SD = Standard deviation

Table 2

EFFECT OF AMMONIUM SULPHATE ON UREA LEVELS (µ moles of urea /g wet wt. of tissues) IN THE TISSUES OF BRAIN, LIVER AND KIDNEY OF MALE AND FEMALE RATS AND LITTERS

Tissues	Adults				Litters	
	Males		Females		Control	Experimental
	Control	Experimental	Control	Experimental		
Brain M	0.648	0.246	0.742	0.348	0.245	0.428
SD	±0.26	±0.18	±0.42	±0.28	±0.24	±0.38
PC		+62.03		+53.09		+74.69
Liver M	7.46	10.38	6.48	9.46	3.72	4.94
SD	±0.42	±0.28	±0.72	±0.82	±0.62	±0.28
PC		+39.91		+45.98		+32.79
Kidney M	35.64	43.62	38.42	52.32	24.28	28.42
SD	±2.82	±1.42	±2.64	±2.64	±1.46	±1.78
PC		+22.40		+36.17		+17.05

All the values are mean ± SD of 6 individual observations.

Free amino acid levels were elevated in both liver and kidney tissues exposed to ammonium sulphate (Table 3). The elevation in free amino acids was in consonance with the increased proteolytic activity. The elevated free amino acid levels indicate the altered protein homeostasis and nitrogen balance due to ammonium sulphate

Table 3

EFFECT OF AMMONIUM SULPHATE ON FREE AMINO ACIDS (µ moles of tyrosine /g wet wt. of tissues) IN THE TISSUES OF BRAIN, LIVER AND KIDNEY OF MALE AND FEMALE RATS AND LITTERS

Tissues		Adults				Litters	
		Males		Females		Control	Experimental
		Control	Experimental	Control	Experimental		
Brain	M	88.42	56.42	85.42	64.35	82.46	68.48
	SD	±6.45	±3.45		±6.38	±5.24	±4.26
	PC		-36.19	±7.26	-24.66		-16.95
Liver	M	15.46	17.64	14.24	18.46	10.26	12.42
	SD	±1.17	±1.28	±1.26	±1.28	±1.28	±1.42
	PC		+14.10		+26.66		+17.93
Kidney	M	108.42	141.18	104.25	130. 4	86.24	100.46
	SD	±6.24	±2.42	±6.24	±3.41	±06.28	±5.28
	PC		+30.65		+24.77		+15.97

All the values are mean ± SD of 6 individual observations.

The ammonia levels were increased in brain, liver and kidney of male, female and litters as shown in Table 4.

Table 4

EFFECT OF AMMONIUM SULFATE ON AMMONIA LEVELS (µ moles of ammonia /g wet wt. of tissues) IN THE TISSUES OF BRAIN, LIVER AND KIDNEY OF MALE AND FEMALE RATS AND LITTERS

Tissues		Adults				Litters	
		Males		Females		Control	Experimental
		Control	Experimental	Control	Experimental		
Brain	M	0.842	1.245	0.792	1.128	0.564	0.742
	SD	±0.042	±0.025	±0.036	±0.46	±0.028	±0.046
	PC		+47.86		+42.42		+31.56
Liver	M	5.46	6.25	5.84	6.48	5.24	6.84
	SD	±0.38	±0.46	±0.42	±0.48	±0.28	±0.36
	PC		+14.46		+10.95		+30.53
Kidney	M	3.68	4.26	3.62	4.76	3.24	4.28
	SD	±0.17	±0.46	±0.24	±0.48	±0.26	±0.38
	PC		+15.84		+31.49		+32.09

All the values are mean ± SD of 6 individual observations.

From Table 5 it was observed that the arginase activity levels increased in brain of female rats and litters whereas they were decreased in the brain of male. Arginase activity levels were greatly decreased in kidney of male and female compared to the litters. The arginase activity levels were increased in liver of female albino rats but they were decreased in male rats and litters.

Table 5

EFFECT OF AMMONIUM SULPHATE ON ARGINASE ACTIVITY LEVELS (µ mole of urea formed /g wet wt. of tissues) IN THE TISSUES OF BRAIN, LIVER AND KIDNEY OF MALE AND FEMALE RATS AND LITTERS

Tissues	Adults				Litters	
	Males		Females		Control	Experimental
	Control	Experimental	Control	Experimental		
Brain M	0.746	0.534	0.678	0.926	0.426	0.646
SD	±0.28	±0.42	±0.32	±0.26	±0.24	±0.18
PC		-28.41		+36.57		+51.64
Liver M	12.48	10.28	11.28	13.46	5.42	3.48
SD	±0.84	±0.62	±0.28	±0.42	±0.18	±0.26
PC		-17.62		+19.75		-35.79
Kidney M	1.72	0.948	1.92	0.842	0.842	0.928
SD	±0.04	±0.02	±0.06	±0.03	±0.04	±0.02
PC		-44.88		-56.14		+10.21

All the values are mean ± SD of 6 individual observations.

The increase of ammonia, urea and arginase activity levels in the tissues of ammonia exposed albino rat and litters suggests the formation of more ammonia resulting in the various deamination processes during which the ammonia so formed may be converted into urea which increases in all tissues studied under ammonia stress. Increased arginase activity in the tissues of albino rats and litters under ammonia stress support the above conclusion drawn.Despite the toxic effects on exposure to sub lethal dose of ammonium sulphate, the rat tries to withstand the toxic effects imposed by the fertilizer by the modulating their physiological and metabolic response towards proper utilization of proteins, free amino acids and ammonia for synthetic processes.From these observations made in rats under ammonia intoxication, it is concluded that the changes are dependent on the dose of fertilizer.

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