

## AMINO ACID CHANGES IN RAT TESTIS IN MANGANESE POISONING

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Rats were given manganese chloride (8 mg/kg) intraperitoneally daily. Manganese content and various amino acids were estimated in the testis at 30 and 60 days. In this period manganese content of the testis showed a 4- and 9-fold increase respectively. Amino acids remained unaltered at 30 days while at 60 days, the amounts of alanine, cysteine, leucine, proline, phenylalanine and glutamine were significantly greater than the levels in the control animals. The present experiments show a great affinity of testicular tissue for manganese resulting in biochemical alterations.

In an earlier communication (1) cellular damage was reported in the testis of rats as a result of intraperitoneal administration of manganese chloride. The results indicated an appreciable damage to the seminiferous tubules at 180 days, to the extent of 50 percent degeneration of the tubules. The present investigation is an attempt to understand the histopathological changes in biochemical terms, namely, the free amino acid changes and its relationship to the manganese concentration in the testis.

### MATERIALS AND METHODS

Male albino rats (average weight 120 g) from I.T.R.C. Colony were used. They were fed a standard diet (contents of calcium and phosphorus in this diet per rat per day were 34.15 and 80.67 mg respectively. Calcium and phosphorus ratio was 1:2.36 with only traces of manganese).

Ninety rats were divided into two groups. Group I consisted of 60 animals which were given a daily dose of manganese chloride (8 mg/kg) intraperitoneally. This dose was calculated on the basis of the experiments performed in this laboratory (2). Group II consisted of 30 rats which were used as controls and received intraperitoneal injections of normal saline.

Twelve rats from group I and six from group II were sacrificed at an interval of 30 and 60 days, respectively. The testes were removed, dried with filter paper, weighed and processed for amino acid analysis. Samples of testes for manganese estimation were stored in a deep freeze ( $-32^{\circ}\text{C}$ ) and later processed. The remaining animals were used for histopathological examination (unpublished data).

Identification and estimation of various amino acids was made according to Kar and Dikshith (3) using two-dimensional paper chromatography. The manganese content of the testes was determined according to the slightly modified method of Srivastava, Pandya and Zaidi (4). Tissues were ashed in a platinum crucible at  $850^{\circ}\text{C}$  for 4 hours (5) in a muffle furnace. Aliquots for manganese estimation were chosen so that the final readings showed a linear relationship.

## RESULTS

Table 1 describes the quantitative changes in eleven free amino acids and manganese content in rat testis as a result of daily intraperitoneal injection of manganese chloride during a period of 30 and 60 days respectively.

Table 1.

*Changes in free amino acids and manganese content in rat testis after intraperitoneal administration of manganese chloride (each figure represents the mean value of 12 rats [ $\pm$  SD] expressed as  $\mu\text{g/g}$  wet weight)*

Amino Acid	Control	Experimental	
		30 days	60 days
Alanine	265 $\pm$ 27	277 $\pm$ 34	320 $\pm$ 30*
Aspartic Acid	480 $\pm$ 71	433 $\pm$ 67	502 $\pm$ 83
Cysteine	188 $\pm$ 88	188 $\pm$ 30	319 $\pm$ 58*
Glycine + Threonine	1299 $\pm$ 93	1292 $\pm$ 46	1344 $\pm$ 32
Leucine	253 $\pm$ 71	205 $\pm$ 14	507 $\pm$ 27*
Proline	706 $\pm$ 94	650 $\pm$ 40	1021 $\pm$ 68*
Phenylalanine	500 $\pm$ 66	449 $\pm$ 83	750 $\pm$ 62*
Serine	700 $\pm$ 84	710 $\pm$ 59	758 $\pm$ 67
Valine	271 $\pm$ 49	247 $\pm$ 76	305 $\pm$ 43
Glutamine	736 $\pm$ 60	736 $\pm$ 60	805 $\pm$ 46*
Lysine	296 $\pm$ 62	296 $\pm$ 62	233 $\pm$ 46
Manganese	2.67 $\pm$ 1.29	10.58 $\pm$ 3.42	27.51 $\pm$ 5.95

\* Results were found to be significantly higher than in controls at 5% level.

The manganese content of the testis showed a 4- and 9-fold increase at 30 and 60 days respectively, indicating rather a greater affinity for the testis in comparison with the brain (2). The levels of various amino acids were found to be unaltered during an experimental period of 30 days. A statistical analysis of the results of free amino acids determinations at 60 days showed, that at 5% level of significance, the amounts

of alanine, cysteine, leucine, proline, phenylalanine and glutamine were greater than the levels in the control group. The levels of aspartic acid, glycine, threonine, serine, valine and lysine remained unchanged.

#### DISCUSSION

The testes have a high cell turnover during the reproductive period and are susceptible to a wide variety of stimuli (6-11). It has been shown that the testes are also highly sensitive to the action of manganese, and the early pathological changes observed are on the spermatogenic epithelium at 150 days (1).

In the present investigation manganese concentration in testicular tissue in rats treated with manganese chloride showed a 4- and 9-fold increase at 30 and 60 days respectively. Along with this rise a significant rise was observed in the contents of alanine, cysteine, leucine, proline, phenylalanine and glutamine at a 60-day period. Lysine however showed no increase.

The present investigations are suggestive of an altered metabolism of the testicular tissue much earlier than the appearance of histological changes. Manganese may be directly responsible for alterations in free amino acids by being a protoplasmic inhibitor of various enzyme systems and thereby limiting the utilization of various amino acids which resulted in an increase. Further investigations are required to find out whether manganese has a direct effect on spermatogenic epithelium or ing effect on testicular tissue.

In humans sexual impotence is a most common manifestation of manganese poisoning which occurs fairly early in the disease process (12, 13). The present experiments show a great affinity of testicular tissue for manganese resulting in biochemical alterations. It may be suggested that in chronic poisoning structural changes may lead to sterility in males.

#### References

1. Chandra, Satya U.: *Acta Pharmacol. Toxicol.*, 29 (1971) 75.
2. Chandra, Satya V., Srivastava, S. P.: *Acta Pharmacol. Toxicol.*, 20 (1970) 177.
3. Kar, P. P., Dikshith, T. S. S.: *Experientia*, 26 (1970) 634.
4. Srivastava, S. P., Pandya, K. P., Zaidi, S. H.: *Analyst.*, 4 (1969) 823.
5. Kun, E.: *J. Biol. Chem.*, 170 (1947) 509.
6. Adamstone, F. B., Spector, H.: *Arch. Path.*, 49 (1950) 173.
7. Kaufman, N., Klavins, J. V., Kinney, T. D.: *Amer. J. Path.*, 312 (1956) 105.
8. Steinberger, E., Dixon, W. J.: *Fertil. Steril.*, 10 (1959) 578.
9. Drobeck, H. P., Coulston, F.: *Arch. Path.*, 81 (1967) 376.
10. Parizek, J.: *J. Endocrinol.*, 15 (1957) 56.
11. Elcoate, P. U., Fischer, M. L., Mawson, C. A., Miller, M. J.: *J. Physiol.*, 129 (1955) 53.
12. Penalver, R.: *Ind. Med. Surg.*, 24 (1955) 1.
13. Balaini, S. G., Umarji, G. M., Bellare, R. A., Merchant, H. C.: *J. Postgraduate Med.*, 13 (1967) 166.

*Sažetak*PROMJENE SADRŽAJA AMINOKISELINA U TESTISU  
ŠTAKORA TROVANIH MANGANOM

Nakon intraperitonealnih aplikacija manganova klorida štakorima u dnevnim dozama od 8 mg/kg, određivan je sadržaj mangana i različitih aminokiselina u testisu. Nakon 30-dnevne aplikacije sadržaj mangana bio je 4 puta veći, a nakon 60-dnevne 9 puta veći nego u kontrolnih životinja. Sadržaj aminokiselina pak nije se bitno promijenio 30 dana nakon tretiranja, ali nakon 60 dana količina alanina, cisteina, leucina, prolina, fenilalanina i glutamina bila je statistički značajno veća nego u kontrolnih životinja.

Ovi pokusi upozoravaju na veliki afinitet tkiva testisa prema manganu, što dovodi do biokemijskih promjena.

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