

INFLUENCE OF ANDROGENS ON URINARY EXCRETION OF 17-HYDROXY STEROIDS AND AMINO ACIDS IN MALE RATS*

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ABSTRACT

Studies were carried out in male albino rats on the influence of age, castration and substitution by testosterone on urinary amino acid excretion pattern and excretion of 17-hydroxy corticoids in the urine.

While age had no effect on amino acid excretion, corticoid activity decreased with age.

Castration caused increased excretion of amino acid and slight increase in corticoid excretion, testosterone produced nitrogen retention and decrease in corticosteroid excretion.

INTRODUCTION

THE recent rapid extension of knowledge of chemistry, biochemistry and physiology of steroid hormones has resulted in increasing attention being focussed upon these substances as regulators of biological processes. In addition to the action on specific sex tissues and other glands of internal secretion, androgens also affect general body processes such as growth, metabolism of protein, adrenocortical activity, etc. Most of present knowledge shows that androgens cause calcium, phosphorus and nitrogen retention. Research on anabolic effect of androgens and catabolic effect of corticoids in nitrogen balance study received tremendous stimulus when therapeutic efficiency of compounds of 19 nor series and cortisone were discovered.

Long, Katzin and Fry (1940) gave a clear demonstration of the role of the adrenal cortex in protein metabolism. These investigators showed that the administration of C-11 oxygenated corticosteroids caused an increase in nitrogen excretion of fasted adrenalectomized rats suggesting that the adrenal cortex exerts some controlling and integrating influence

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on both protein and carbohydrate metabolism. Since then there has been a great deal of interest and activity in this field. Group of research workers have experimented to establish that biochemical disorders are related to steroid hormones. It has been known that small amounts of amino acids and 17-hydroxy corticosteroids are excreted in urine and the levels can be altered with the metabolic changes. The further recent rapid extension of knowledge of steroid hormones has set a stage for enquiry into the relationship of steroid hormones and protein metabolism. Studies were therefore undertaken to see the changes that occur in free alpha amino acid excretion and 17-hydroxy corticoids excretion during castration and steroid treatment, and to examine the correlation between them.

MATERIAL AND METHODS

Parts of the studies related to observing these changes by experimenting on male rats. The 17-hydroxy corticosteroids excretion was studied quantitatively, the amino acids excretion was studied both qualitatively as well as quantitatively. James—Caie method was used for study of 17-hydroxy corticosteroids excretion. The qualitative study of alpha amino acid excretion was carried out by using two-dimensional paper chromatography technique and the quantitative study of free alpha nitrogen level was done by using colorimetric (ninhydrin) method.

Albino rats of local variety were used and were divided into various groups (Tables I, II and III). 24 hours urine was collected under constant food intake and water was allowed freely. 0.5 ml. toluene was used as a preservative.

RESULTS

The values obtained for alpha amino nitrogen, 17-hydroxy corticoids and normal alpha amino acid pattern in male rats are represented in Table I.

Table II shows free alpha amino nitrogen excretion in sham-operated, castrated and testosterone-treated rats.

Table III shows excretion of 17-hydroxy corticoids in sham-operated, castrated and testosterone-treated rats.

Lysine, glycine, serine, threonine, alanine, leucine and/or isoleucine are excreted in all groups of male rats. Amino acid pattern does not change after sham operation. Castration produces aminoaciduria, but no definite pattern is observed. Testosterone treatment maintains amino acid pattern similar to normal during the treatment. Serine is excreted after sham

TABLE I

Influence of age on excretion of alpha amino nitrogen, 17-OH corticoids and free alpha amino acid pattern in normal male rats

Group No.	No. of animals	Av. body weight (g.)	Alpha amino N ₂ excretion mgm./50 ml.	17 OHCS excretion μ gm./24 hrs.	Free alpha amino acids excreted in urine					
					Lysine	Glycine	Serine	Threonine	Alanine	Leucine and/or isoleucine
I	6	67.0 \pm 3.31	14.4 \pm 3.61	12.20 \pm 1.38	+	+	+	+	+	+
II	6	123.6 \pm 4.8	17.6 \pm 5.1	4.91 \pm 1.48	+	+	+	+	+	+
III	6	221.6 \pm 10.41	17.2 \pm 3.80	9.60 \pm 2.08	+	+	+	+	+	+

TABLE II

Effect of male sex hormone on free alpha amino nitrogen excreted by male rats on different days after castration

Mode of treatment	Average body weight in g.	Normal Amino N ₂ excretion mgm./50 ml.	Free alpha amino nitrogen excreted on different days per 50 ml. of urine in mgm.*										
			Number of days										
			1	2	3	4	5	6	7	8	9	10	11
Sham operation	134.0 \pm 13.71	17.9 \pm 4.30	58.4 \pm 10.85	37.7 \pm 7.74	30.7 \pm 5.37	23.5 \pm 5.21	20.5 \pm 1.27	19.2 \pm 1.17	18.4 \pm 1.00
Castration	139.0 \pm 12.20	17.2 \pm 5.61	60.0 \pm 14.3	72.3 \pm 14.2	62.4 \pm 15.5	46.4 \pm 11.85	33.7 \pm 5.20	25.3 \pm 5.02	18.0 \pm 0.826
Castration + Testosterone propionate	153.6 \pm 13.32	..	25.0 \pm 3.86	30.6 \pm 3.89	27.0 \pm 3.19	22.2 \pm 2.29	22.1 \pm 4.7	19.6 \pm 0.864	17.15 \pm 1.38	23.7 \pm 1.92	39.3 \pm 4.87	30.5 \pm 5.90	25.9 \pm 3.97

* Number of animals treated = 6.

operation and after castration, whereas it disappears after testosterone treatment. Phenylalanine is excreted only in sham-operated and testosterone-treated rats. Phenylalanine excretion is not observed in castrated rats.

In sham-operated male rats, both amino nitrogen excretion as well as total 17-hydroxy corticoid excretion level increase on the first day of opera-

TABLE III

Effect of male sex hormone on 17-hydroxy corticoids excreted by male rats on different days after castration

Mode of treatment	Av. body weight in g.	Normal 17-OHCS $\mu\text{gm./24 hrs.}$	17-Hydroxy corticoid excreted in $\mu\text{gm./24 hours}^*$										
			Number of days										
			1	2	3	4	5	6	7	8	9	10	11
Sham operation	134.0 ± 13.71	6.73 ± 0.82	15.23 ± 6.19	7.98 ± 1.34	11.59 ± 2.08	17.30 ± 2.92	7.34 ± 2.04	6.24 ± 1.43	6.08 ± 1.40
Castration	139.0 ± 12.20	5.62 ± 0.99	6.13 ± 1.37	4.81 ± 1.13	5.22 ± 0.86	5.73 ± 1.09	5.83 ± 1.43	6.25 ± 1.19	5.40 ± 0.90
Castration + Testosterone propionate	153.6 ± 13.32	..	5.44 ± 1.08	4.74 ± 0.72	4.47 ± 0.96	4.06 ± 1.02	3.75 ± 0.89	3.50 ± 0.91	4.11 ± 0.39	4.10 ± 0.84	3.82 ± 0.80	4.37 ± 0.67	4.09 ± 0.75

* Number of animals treated = 6.

tion. Both the levels return to normal on the 5th day. There is a significant increase in the level of amino nitrogen excretion and a slight increase in the 17-hydroxy excretion level in castrated rats. Testosterone propionate produce nitrogen retention and lowers corticoid activity.

DISCUSSION

In the present study it is observed that age has no effect on amino acid pattern in male rats. Lysine, glycine, serine, threonine, alanine, leucine and/or isoleucine are excreted in all groups of male rats. Present finding regarding the effect of age is consistent with David Seligson's (1950) observation. In the present study it has been further seen that corticoid activity declines with age. This finding is supported by studies on aging by Samuel (1962). From the recent studies carried out by Espiner (1966), it has been seen that there is a 10-40-fold increase in the urinary excretion of biologically active steroids after major operation. Present investigations show increase in levels of alpha amino nitrogen and corticoids after sham operation, probably due to post-traumatic metabolic changes caused by the stress of operation.

Castration in males is followed by an increased size of adrenals. Pearson and Eliel (1952) have reported that testosterone would counteract some of the effects of cortisone on normal tissue. Butler *et al.* (1953) gave testosterone to two adrenalectomized patients maintained on 25-50 mg. of cortisone

daily. Gamzell and Notter (1956) have observed that the adrenalectomized patients treated with testosterone propionate in a dosage of 50 mg. daily for three days caused a decrease in the urinary corticoids from about 35 mg to 13 mg. and that two days after the last injection of testosterone propionate the urinary corticoids returned to preinjection values. All these authors have also considered an indirect effect of androgens on adrenal cortex via the pituitary. Data are scarce regarding the effect of castration and gonadal hormone on corticoid activity. However, it has been reported by Rindani (1957) that castration partially inhibited stress induced activation of adrenal cortex and that this modification of the response of the gland was prevented by the administration of testosterone to the castrated animals. The slight increase in corticoid levels in the castrated animals may be explained on the basis of absence of inhibitory effect of testosterone after castration. Decreased activity after testosterone administration may be suggestive of inhibitory effect of gonadal hormone.

The influence of androgens on protein metabolism has been studied in detail since the demonstration by Kochakian and Murlin (1935) that testosterone is capable of causing nitrogen retention in castrated dogs. Verifications of this finding were made by many workers but very little attention has been paid specifically to the free alpha amino acid excretion. In the present study it was noticed that castration induces aminoaciduria though no definite pattern of amino acid was observed, while testosterone propionate produces amino nitrogen retention.

It has already been demonstrated by Zinneman *et al.* (1963) that cortisol administration enhances the urinary excretion of serine, threonine, alanine, asparagine, glutamine, histidine, glycine, cystine, phenylalanine, lysine, cystathionine, valine, alpha *n*-butyric acid, leucine and ornithine. The present study of testosterone therapy shows that the retention is limited to the same group of amino acids, *i.e.*, serine, glycine, alanine, threonine, lysine, leucine and/or isoleucine. Comparing testosterone with cortisol it is found that both act on the same group of amino acids. Cortisol produces increased excretion of this group of amino acids whereas testosterone produces retention in the same group of amino acids. Renal clearance studies of cortisol indicate that increased excretion of the amino acids may be due to a defect in renal tubular reabsorption but in the case of testosterone such a study has not been carried out.

SUMMARY

1. Age has no effect on amino acid excretion while corticoid activity decreases along with the age in male rats.

2. Lysine, glycine, serine, threonine, alanine, leucine and or isoleucine are excreted in all groups of male rats.

3. In sham-operated rats both amino nitrogen excretion as well as total 17-hydroxy corticoid output in the urine increase on the first day of the operation. Both the levels return to normal on the fifth day of urine collection. It may probably be due to protein catabolic action of corticoids which are increased due to stress of sham operation.

4. In the case of castrated male rats amino nitrogen excretion increases. Castration induces aminoaciduria. Corticoid excretion slightly increases in castrated male rats.

5. Testosterone propionate in male rats produces nitrogen retention probably due to anabolic action of testosterone propionate. It lowers the adrenal cortical activity, perhaps due to inhibitory effect of testosterone on adrenal cortex.

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INDEX TO VOL. LXVIII (B)

AUTHORS' INDEX

- Agnihotrudu, V. .. *Phaeodactylium venkatesanum* Gen. et sp. nov.
on *Elettaria cardamomum* Mat. from Kerala,
206.
- Basu, A. N. .. See Rao and others.
- Bhatnagar, G. C. and Prasad, N. Utilization of carbon compounds by *Fusarium solani* F. *aurantifoliae* Bhat. and Prasad, 163.
.. Effect of micro-nutrients on the growth and sporulation of *Fusarium solani* F. *aurantifoliae* Bhat. and Prasad, 169.
- Biradar, N. V. and Mahabale, T. S. .. Studies on palms: embryology of *Phoenix robusta* Hook, 1.
- Chacko, M. J. .. See Rao and others.
- Chandra, Prakash and Nayar, B. K. .. Morphology of the edible fern *Ampelopteris* Kunze, 25.
- Durve, V. S. .. Some observations on the conditioned responses in the fingerlings of the Milk fish *Chanos chanos* (Forskål), 69.
- Ghosh, R. K. .. See Mukherjee and Ghosh.
- Govindarajalu, E. .. Further contribution to the anatomy of the Marantaceae: *Schumannianthus virgatus* (Roxb.) Rolfe, 250.
- Hrishi, N. and Marimuthammal, S. Studies in mutagenesis in sugarcane, I, 131.
.. Embryo culture of maize and sugarcane hybrids, 308.
- Hrishi, N., Krishnamurthy, T. N. and Marimuthammal, S. Studies in mutagenesis in sugarcane, II, 181.
- Kamble, N. P. .. See Subramanyam and Kamble.

- Krishnamurthy, T. N. .. See Hrishi and others.
- Lal, Bihari and Tandon, R. N. Utilization and synthesis of oligosaccharides by some pathogenic isolates of *Colletotrichum capsici* (Syd.), Butler and Bisby, 269.
- Madhavikutti, K. and Shanmugasundaram, E. R. B. Toxicity of *Aspergillus flavus* ex Link Fries and *Penicillium rubrum* Stoll to Swiss Albino mice, 261.
- Mahabale, T. S. .. See Biradar and Mahabale.
- Mahadevappa, M. .. Studies on heterosis in pearl millet (*Pennisetum typhoides* Stapf and Hubb), III, IV, 190, 210.
- Mandahar, C. L. .. See Thind and Mandahar.
- Marimuthammal, S. .. See Hrishi and Marimuthammal.
.. See Hirshi and others.
- Mukherjee, R. P. and Ghosh, R. K. On the synonymy of the genus *Artyfechinostomum* Lane, 1915 (Trematoda: Echinostomatidae), 52.
- Murthy, N. B. Krishna .. See Reddy and Murthy.
- Nambiar, K. K. N. and Ramakrishnan, K. Studies on pigeon pea sterility mosaic disease, VI, 295.
- Narkhede, M. N. .. See Phadnis and others.
- Nayar, B. K. .. See Chandra and Nayar.
- Nayar, M. P. .. An interesting new species of *Phyllagathis* Bl. (Melastomataceae), 301.
- Pai, R. M. .. See Tilak and Pai.
- Pandotra, V. R. and Sastry, K. S. M. Studies on Sclerotium rot of mints, 9.
- Parthasarathy, M. D. .. See Rahaman and Parthasarathy.
- Parvatheswararao, V. .. Initial adaptive responses to thermal stress in freshwater teleosts, I, 225.
- Pasha, S. M. Kamal .. Studies on the exocrine pancreatic tissue of three teleosts, 289.
- Phadnis, B. A., Narkhede, M. N. and Thombre, M. V. Further studies in polysomaty in root-tips of *Cicer arietinum*, Linn., 279.
- Phalak, V. R. .. See Rao and others.
- Prasad, N. .. See Bhatnagar and Praşad.
- Ponnappa, K. M. .. Some interesting fungi, III, 175.

- Rahaman, Hafeezur and Parthasarathy, M. D. The chromosome complement of the Bonnet monkey (*Macaca radiata*) determined in leucocytes, 143.
- Ramakrishnan, K. .. See Nambiar and Ramakrishnan.
- Rao, C. Venkata .. Studies in the Proteaceae, XI, 11.
- Rao, H. Dinesh .. See Rao and others.
- Rao, V. P., Basu, A. N., Phalak, V. R., Chacko, M. J. and Rao, H. Dinesh Some new records of parasites of rice stem-borers in India, 91.
- Rawal, U. M. .. Anatomy of the feeding apparatus of Hoopoe—*Upupa epops epops* Linnaeus, 79.
- Reddy, G. Sreerama and Murthy, N. B. Krishna *Drosophila rajasekari*—a new species from Mysore (India), 202.
- Rindani, R. H. .. See Tipnis and Rindani.
- Sastry, K. S. M. .. See Pandotra and Sastry.
- Shah, J. J, and Unnikrishnan, K. Vascular connections of axillary buds in some members of the Verbenaceae, 284.
- Shanmugasundaram, E. R. B. See Madhavikutti and Shanmugasundaram.
- Shenoy, M. A., Srinivasan, V. T. and Singh, B. B. Radiochemical basis of sensitisation of *Staphylococcus aureus* by Vitamin K₅, 232.
- Singh, B. B. .. See Shenoy and others.
- Srinivasan, K. V. .. Effect of a varietal mixture on sugarcane root rot susceptibility and on the rhizosphere microflora, 150.
- Srinivasan, V. T. .. See Shenoy and others.
- Sudarsan, D. .. Brachiopod larvae from the west coast of India, 59.
- Subramanian, C. V. and Tyagi, P. D. Utilization of various forms of nitrogen by *Drechslera sorokiniana*, the pathogen causing foot rot disease in wheat, 111.
- Subramanyam, K. and Kamble, N. P. Chromosome numbers in certain Indian species of *Utricularia* L. (Lentibulariaceae), 221.
- Tandon, R. N. .. See Lal and Tandon.
- Thind, K. S. and Mandahar, C. L. Trace element studies on some pathogenic fungi, 37.
- Thombre, M. V. .. See Phadnis and others.
- Tilak, V. D. and Pai, R. M. Studies in the floral morphology of the Marantaceae, II, 240.

- Tipnis, P. A. and Rindani, R. H. Influence of androgens on urinary excretion of 17 hydroxy steroids and amino acids in male rats, 314.
- Tyagi, P. D. .. See Subramanian and Tyagi.
- Unnikrishnan, K. .. See Shah and Unnikrishnan.
- Venkataramani, K. S. .. See Venkata Ram and Venkataramani.
- Venkata Ram, C. S. and Venkataramani, K. S. Blister-like malformations on tea seedlings, 304.

TITLE INDEX

- Adiantum species* Kunze, edible fern, morphology of the (Chandra and Nayar), 25.
- Athyfechinostomum* Lane, 1915 (Trematoda: Echinostomatidae), the genus, on the synonymy of the (Mukherjee and Ghosh), 52.
- Aspergillus flavus* ex Link Fries and *Penicillium rubrum* Stoll, toxicity of, to Swiss Albino mice (Madhavikutti and Shanmugasundaram), 261.
- Orangutan monkey (*Macaca radiata*), the chromosome complement of the, determined in leucocytes (Rahaman and Parthasarathy), 143.
- Isopod larvae from the West Coast of India (Sudarsan), 59.
- Mastomys chanos* (Forskål), the milk fish, conditioned responses in the fingerlings of, some observations on (Durve), 69.
- Micrococcus arietinum*, Linn., root-tips of, further studies in polysomaty in (Phadnis and others), 279.
- Polletotrichum capsici* (Syd.) Butler and Bisby, some pathogenic isolates of, utilization and synthesis of oligosaccharides by (Lal and Tandon), 269.
- Reclisera sorokiniana*, the pathogen causing foot rot disease in wheat, utilization of various forms of nitrogen by (Subramanian and Tyagi), 111.
- Rosophilula rajasekari*—a new species from Mysore (India), (Reddy and Murthy), 202.
- Fungi, some interesting, III (Ponnappa), 175.
- Fungi, some pathogenic, trace element studies on (Thind and Mandahar), 37.
- Sclerotium solani* F. *aurantifoliae* Bhat. and Prasad, effect of micro-nutrients on the growth and sporulation of (Bhatnagar and Prasad), 169.
- Sclerotium solani* F. *aurantifoliae* Bhat. and Prasad, utilization of carbon compounds by (Bhatnagar and Prasad), 163.
- Scorpion, *Upupa epops epops* Linnaeus, anatomy of the feeding apparatus of (Rawal), 79.
- Maize and sugarcane hybrids, embryo culture of (Hrishi and Marimuthammal), 308.
- Marantaceae: *Schumannianthus virgatus* (Roxb.) Rolfe, further contribution to the anatomy of the (Govindarajalu), 250.
- Marantaceae, studies in the floral morphology of the, II (Tilak and Pai), 240.
- Palms, studies on: Embryology of *Phoenix robusta* Hook (Biradar and Mahabale), 1.
- Parasites of rice stem-borers in India, some new records of (Rao and others), 91.

- Pearl millet (*Pennisetum typhoides* Stapf and Hubb.), studies on heterosis, III, IV (Mahadevappa), 190, 210.
- Phaeodactylum venkatesanum* Gen. et sp. nov. on *Elettaria cardamomum* Mat. from Kerala (Agnihotrudu), 206.
- Phyllagathis* Bl. (Melastomataceae), an interesting new species of (Nayar), 301.
- Pigeon pea sterility mosaic disease, studies on, VI (Nambiar and Ramakrishnan), 295.
- Proteaceae, studies in the, XI (Rao), 11.
- Rats, male, influence of androgens on urinary excretion of 17 hydroxy steroids and amino acids in (Tipnis and Rindani), 314.
- Sclerotium rot of mints, studies on (Pandotra and Sastry), 9.
- Staphylococcus aureus*, radiochemical basis of sensitisation of, by Vitamin K₅ (Shenoy and others), 232.
- Sugarcane root rot susceptibility and the rhizosphere microflora, effect of a varietal mixture on (Srinivasan), 150.
- Sugarcane, studies in mutagenesis in, I (Hrishi and Marimuthammal), II (Hrishi and others), 131, 181.
- Tea seedlings, blister-like malformations on (Venkata Ram and Venkataramani), 304.
- Teleosts, freshwater, initial adaptive responses to thermal stress in (Parvatheswararao), 225.
- Teleosts, three, studies on the exocrine pancreatic tissue of (Pasha), 289.
- Utricularia* L. (Lentibulariaceae), certain Indian species of, chromosome numbers in (Subramanyam and Kamble), 221.
- Verbenaceae and the Labiatae, some members of the, vascular connections of axillary buds in (Shah and Unnikrishnan), 284.