THE EFFECT OF TESTOSTERONE PROPIONATE ON INDUCED CREATINURIA IN RATS*

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That the gonads may be involved in creatine and creatinine metabolism was indicated when Rose (1) discovered a persistent creatinuria in children before puberty. After puberty the creatinuria ceases in boys, but continues to a lesser extent or in a cyclic manner in girls and women. In support of this finding, Read (2), McNeal (3), Remen (4), Bühler (5), Pizzolato and Beard (6), and others have claimed that castration in humans and animals leads to a creatinuria. In contrast, Tsun-Chee Shen (7), Kochakian and Murlin (8), Sandberg, Perla, and Holly (9), and others have not observed an induced creatinuria in men, dogs, or rats by castration. Considerable difference of opinion also exists concerning the effects of sex hormones upon creatine and creatinine excretion in hypogonadism and following castration. Bühler (10), Kun and Peczenik (11), Paschkis and Schwoner (12), and Kenyon et al. (13) find that androgens effect a decrease in hypogonad creatinuria. On the other hand, Pizzolato and Beard (6) report that castration in rats produces a creatinuria which is not decreased, but actually is increased by testosterone propionate administration.

In view of the many conflicting reports an investigation was undertaken on urinary excretion of creatine and creatinine in normal and castrated male rats kept on a creatine- and creatinine-free, high protein diet with and without testosterone propionate treatment and a repetition with a controlled intake of creatine.

EXPERIMENTAL

Equipment and Methods—The Dubos-Miller modification (14) of the Folin procedure (15) was slightly altered and adapted for

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use in the Evelyn photoelectric colorimeter. The combination of light filters, Rubicon No. 4785 and Wratten No. 75, cemented in “C” glass, permits the maximum transmission of light in the 490 to 500 mp range. This is the most selective range for the Jaffe picrate reaction.

It was found necessary to use the bright light of the instrument. As a result a slightly different manipulation was adopted than when the dim lamp is used. A blank solution containing 1.0 cc. of 2 N HCl, 1.0 cc. of 2 N NaOH, 8.0 cc. of water, and 5.0 cc. of alkaline picrate was allowed to stand for 10 minutes after the addition of the alkaline picrate. Meanwhile the proper light filters had been placed in the colorimeter and the dim lamp turned on. The rheostats were adjusted until the galvanometer read about 55. At the end of the 10 minute period, the colorimeter tube with its contents was placed in the instrument, the bright light turned on, and the rheostats adjusted until the galvanometer read 100. The bright lamp was then turned off, the tube removed, and the new galvanometer reading with the dim lamp observed. This new reading is called the “center reading.” This manipulation was repeated for 1.5 minutes in order to secure a constant center reading with the particular instrument used. This probably is not due to any color change in the solution, but to varying the light intensity and the heat effects arising therefrom. The same procedure of removing the tube to check the center reading was used for all creatinine measurements, and it gave satisfactory and reproducible results over extended periods of time. The advantage of the center reading is that it serves as a reference point of light intensity, and eliminates numerous blanks in a particular set of creatinine determinations. It should be determined with each set of creatinine values.

The reagent picric acid was prepared from Eastman’s No. 210 by purifying twice by Benedict’s method (16). The 1.2 per cent solution was made without the application of heat by trituration with water at room temperature. This solution was freshly prepared 24 hours before each run.

The alkaline picrate solution was made by mixing 5 volumes of the 1.2 per cent picric acid and 1 volume of exactly 2.5 N NaOH and allowing to stand for 15 minutes before use. It was found safe for use up to 6 hours after preparation.
The creatine was converted into creatinine in special conversion tubes. The tubes of 25 cc. capacity were made from No. 24/40 interchangeable ground glass connections and provided with stoppers, No. 24/25. The stoppers and tubes were fitted with glass hooks for the application of springs or wires. For the conversion of creatine to creatinine the standard solution or unknown was measured into the dry tube, water added to dilute to 5 cc., and after the addition of 1 cc. of 2 N HCl the tubes were stoppered, securely wired, and autoclaved at 20 pounds pressure for 45 minutes. The autoclave was allowed to cool until the pressure returned to the atmospheric before it was opened. After the tubes had cooled to room temperature, 1 cc. of 2 N NaOH and 3 cc. of water were added and the mixture stirred with a fine stirring rod. For the color development, 5 cc. of the alkaline picrate solution were added and 10 minutes after thorough stirring with a glass rod the mixture was transferred to the colorimeter tube and read in the colorimeter.

The standard creatinine curve was prepared by measuring the standard creatinine solution directly into the colorimeter tube, adding 1 cc. of 2 N HCl, 1 cc. of 2 N NaOH, and diluting to 10 cc. with water. The acid and base were added in order to have the same salt concentration as in the creatine estimation. The 5 cc. of alkaline picrate were added and 10 minutes after thorough mixing the tubes were read in the colorimeter. Ranges of 0 to 0.05 mg. of creatinine and the equivalent of creatine were run. The standard curve is given in Fig. 1. Obviously Beer's law holds and the creatine is quantitatively converted into creatinine. The accuracy of the curve is ±2.2 per cent as a maximum deviation from the mean in the range given.

Animal Experiments—Sixteen male rats of the same age from our inbred colony were used. Eight were castrated at 3 months of age. All were placed in metabolism cages maintained at 22°. Two rats were kept in each of eight cages on a constant diet for 2 months prior to the urine analysis. The diet consisted of commercial casein 18 per cent, corn-starch 53, inorganic salt mixture (17) 4, butter fat 8, cod liver oil 2, dried brewers' yeast 15. Each day the cages, funnels, and volumetric flasks for urine collection
were thoroughly cleaned at the same hour in an attempt to keep all conditions constant. On urine collection days, the cages, false bottoms, and funnels were washed thoroughly with distilled water. The feces-free washings were collected in a 500 cc. volumetric flask, made up to volume with water, and filtered. Aliquots of the filtrate were taken for creatinine and total creatinine determinations.

After a control period on creatine and creatinine excretion, creatine amounting to 40 mg. per kilo of body weight was administered orally to each rat in two divided doses each day. The creatine solution was administered by a stomach tube, which consisted of a No. 14 1\(\frac{1}{2}\) inch blunt metal tube attached to a hypodermic syringe. The tube was placed down the rat's esophagus and the water solution of creatine forced into the stomach. This allows the creatine to be applied in a manner which simulates that of normal feeding and causes it to follow the normal path of absorption from the intestine. Thus an intense creatinuria was produced. After 5 days of creatine administration the rats approximated a constant body weight. Thereafter, throughout the entire experiment the corresponding amount of creatine was given. Thus, as the rats gained weight the creatine administered fell somewhat below the value of 40 mg. per kilo of body weight.
Creatine alone was given for 15 days until an intense creatinuria was produced. On the 16th day, in addition to the continued ingestion of creatine, each rat was injected daily with 900 \( \gamma \) of testosterone propionate in sesame oil for 14 days. During the androgen injections the urine was pooled and extracted for androgenic assay. This and a similar previous experiment resulted in no androgenic activity being excreted by the normal and castrated rats, although a total of 13,440 r.u. of testosterone propionate had been administered.

After the 14 day period of testosterone propionate and simultaneous creatine administration, both treatments were discontinued and the creatine and creatinine excretion observed for 11 more days.

During the entire experiment the body weights were recorded for correlation studies.

The creatinine and total creatinine determinations were made by diluting the 24 hour specimen of urine to the equivalent of 1 liter, and using 1 cc. aliquots for duplicate analyses. The amount of non-creatinine chromogenic material in the urine was determined before and after autoclaving by the use of the Dubos-Miller (14) specific creatine- and creatinine-destroying cultures. It amounted to approximately 1 mg. of creatinine equivalent per day and was constant before and after autoclaving, thus eliminating the necessity for any corrections to be applied to the total creatinine determination.

Figs. 2 and 3 show the values obtained for the body weights, and creatine and creatinine excretion in normal and castrated rats respectively. In the normal rats the creatinuria during the control period is of a very low order. Creatine administered orally causes intense creatinuria. Testosterone propionate injection during creatine ingestion lowers the creatinuria. Simultaneously with the decrease in creatine excretion there is an increase in body weight which approximates a new high level. After this level is reached, the creatinuria again increases. In the fourth period, when the androgen and creatine administrations were discontinued, there was a slight decrease in body weight and a return of the creatine excretion to the pretreatment level. The values for the castrated rats give a similar set of curves, but the changes in creatinuria and body weight are much greater than in the normal
rats, and the attainment of the new high level in body weight requires about 3 days longer than for the normal animals.

To show more clearly that the castrated rats do excrete less of the ingested creatine than the normals during androgen administration, the average values of creatine excreted are expressed as per cent of creatine fed, and plotted as maximum and minimum deviation from the mean excretion values. Fig. 4 shows these relationships. Although there is a greater retention of ingested creatine by the castrated rats, both types of animals react in a similar fashion to the creatine and androgen treatments. The retention differs quantitatively but not qualitatively. Thus, there is a striking parallelism in the creatine excretion curves. Also the ratio of body weight gains of castrates and normals and the ratio
of the creatine retained are 1.7 and 1.45 respectively. That is, increased body weight paralleled the increased creatine retention.

Subsequent to the urinary creatine investigations, an estimation of muscle creatine was undertaken on the same rats. They were kept on the stock diet for 36 days after the previous urine experiments. At the end of that time the animals were divided into four groups. One set each of normals and castrates received

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<th>40 MGMS. CREATINE PER KG/M. BODY WEIGHT</th>
<th>CONTROL</th>
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<tr>
<td>NO ANDROGEN</td>
<td>WITH ANDROGEN</td>
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**Fig. 3.** The urinary creatine and creatinine from castrated adult male rats. The solid line represents the data for Pair 1; the dash line, Pair 2; the dot and dash line, Pair 5; the cross and dash line, Pair 6. The creatine and creatinine values are expressed as mg. per kilo of body weight.

40 mg. per kilo of body weight of creatine orally each day, while the other sets of normals and castrates received 900 γ of testosterone propionate in sesame oil daily in addition to the ingested creatine. The creatine and androgen were administered for 19 days after which time a constant elevated body weight was reached in the androgen-injected animals. The rats were then sacrificed and the gastrocnemius muscle removed from both hind
The urinary creatine as per cent of creatine fed. The high and low values of the mean deviation from the average are plotted.

**Table I**

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<th>Control</th>
<th>900 γ testosterone propionate daily for 19 days</th>
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<tr>
<td></td>
<td>Castrated</td>
<td>Normal</td>
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<td>Maximum</td>
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<td>Minimum</td>
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<td>Average</td>
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legs for creatine determinations. In order to obtain a representative sample of tissue, the muscles were immediately frozen on
solid carbon dioxide and pulverized in a previously chilled iron mortar. The powdered tissue was stored at \(-5^\circ\) for a short time until aliquots were taken for total creatinine, moisture, and fat determinations. The total creatinine was determined by the method of Miller, Allinson, and Baker (18) by the use of the Dubos-Miller specific creatine- and creatinine-destroying cultures. A standard curve for creatinine concentration versus per cent of light transmission was produced under conditions identical with those used for the tissues. Table I shows the total creatinine values in the gastrocnemius tissue. These figures indicate that there is no significant difference in the muscle creatine between normal and castrated rats with and without androgen administration, but with a liberal supply of exogenous creatine.

**DISCUSSION**

The gain in body weight as a result of androgen therapy is in accordance with the observations of Korenchevsky, Dennison, and Brovain (19) on castrated rats, and Kenyon, Sandiford, Bryan, Knowlton, and Koch (13) in eunuchoids. The latter group believe that from one-seventh to one-half of this gain probably is due to protein being laid down as indicated by the nitrogen retention studies, but that a considerable amount of the increase is due to water and sodium retention. Similarly, Thorn and Harrop (20) have found that sodium and its associated water are retained in the normal dog during the administration of estrone, estradiol, progesterone, pregnanediol, and testosterone.

Gaebler and Bartlett (21) have produced decreased exogenous creatinuria in adult female dogs by the administration of antuitrin growth preparation. Simultaneously, there resulted an increase in nitrogen retention and body weight. Here the decrease in exogenous creatinuria also parallels body weight gain. This may be an instance of a substance other than a sex hormone which has a similar effect on body weight and creatinuria. However, it is possible that the growth hormone preparation which these workers used was not free from gonadotropic factors and hence may have produced the changes through the gonads. The fact that prepubertal growth is associated with a constant creatinuria in both sexes, and that postpubertal growth results in a diminished creatinuria, may be of significance in the interpretation of these results.
Effect of Testosterone Propionate

The belief held by some investigators, that creatine excretion in men is an indication of a hypogonadal function, is not confirmed by the studies in rats. Prior to feeding of creatine no significant difference in creatinuria existed between the normal and castrated rats. During creatine feeding the creatinuria of the castrated rats varied from 12 to 22 mg. per kilo of body weight, whereas the normal rats excreted 20 to 26 mg. per kilo of body weight just prior to androgen injection. Fig. 4 shows that throughout the creatine feeding period the castrated rats tended to excrete less of the ingested creatine than the normal animals.

Another question which requires more investigation is the creatinuria of normal men. It has been generally accepted that normal men do not have a creatinuria, but evidence is accumulating which indicates that this belief is not well founded. Taylor and Chew (22) found from 0 to 196 mg. of creatine nitrogen in the urine of fifteen adult males. Other unpublished observations also indicate that normal men may excrete creatine in the urine in varying amounts.

Since it is generally believed that creatine when ingested is in part stored by the muscles, and about 98 per cent of the body's creatine resides in the musculature, it seems possible that the increased exogenous creatine retention observed in these experiments, paralleling body weight gain, represents an increased muscle tissue production under the influence of testosterone propionate. This view is supported by the observations of Papanicolaou and Falk (23) who showed that the temporal muscles of male guinea pigs are larger than those of females. They also observed that in male and female castrates a muscular hypertrophy was produced by the administration of testosterone.

The observation that the castrated rats had a body weight gain of 1.7 times that of the normals is in agreement with Kochakian and Murlin's (8) reports on the effects of androstenedione upon nitrogen retention in castrated and normal dogs. Their experiments showed very little if any nitrogen retention on the part of the normal as compared with the castrated dog. As a result of this, they felt that an animal with normally functioning gonads is being supplied with sufficient hormone to maintain at least its accessory sex organs in a normal physiological condition, and that further androgen is either not utilized or is met with a compensating set of factors. The doses used in their dogs were low as com-
pared with levels used in the rats. Therefore, in the rats it appears that in the normal animal, although it does gain weight, there is some compensating factor at work which decreases the effect of excess androgen before a similar condition is brought about in the castrated animal. This is indicated by the lower gain in weight which reaches a maximum about 3 days earlier than in the castrates.

**SUMMARY**

The quantitative estimation of creatine and creatinine has been studied by the use of the Jaffe reaction, Evelyn photoelectric colorimeter, and the Dubos-Miller specific creatine- and creatinine-destroying cultures. Certain modifications of the determination have been made and applied to biological materials.

Adult male rats which have been in a castrated condition for 2 months do not develop a distinct creatinuria other than the insignificant and apparent creatinuria existing prior to operation.

Daily injection of 900 γ of testosterone propionate caused an increase in body weight and a decrease in excretion of exogenous creatine. The body weight gain and the decrease in creatinuria were greater in the castrated than in the normal animal.

The castrated and normal animals react in a similar fashion to exogenous creatine and testosterone propionate. During testosterone propionate and creatine administration the changes in creatininuria parallel body weight change until the body weight reaches a high level. At this level an intense creatinuria reappears even though androgen administration is continued together with the ingestion of creatine. When the administration of androgen and creatine is discontinued, the creatinuria falls to the insignificant values of the pretreatment level.

The muscle creatine content of normal and castrated rats with and without androgen treatment, but with a liberal supply of exogenous creatine, shows no significant difference. It would appear that the muscle tissue of the castrated rat is normal with respect to creatine content under the conditions of the experiment.

**BIBLIOGRAPHY**

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