Effect of Androgen on the Incorporation of Orotic Acid-6-^{14}C into the Ribonucleic Acids and Free Nucleotides of Mouse Kidney*

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ABSTRACT: The incorporation of orotic-acid-6-^{14}C into the ribonucleic acid (RNA) of the mouse kidney was rapidly increased after castration to a maximum value of approximately twice that of normal and restored to normal within 7 days by the administration of testosterone propionate. The changes were accompanied by parallel changes in the radioactivity in the acid-soluble fraction (the pool). The concentration of uracil, uridine, uridine monophosphate (UMP), uridine diphosphate (UDP), and uridine triphosphate (UTP) in the acid-soluble fraction was not changed by castration; neither was that of the cytosine, adenine, and guanine compounds.

The majority of the radioactivity, about 90%, was in the uracil compounds. Although UTP was present in the highest concentration, it had a very low specific radioactivity. Castration increased twofold the specific radioactivity of the compounds. Testosterone propionate decreased the specific radioactivity of uracil, maintained that of UTP at the elevated level, and restored that of the other compounds to normal. The concentration of UMP, UDP, and UTP was increased by the androgen while that of the others was maintained at the normal level.

The ribonucleic acids of the mouse kidney are decreased after castration and rapidly increased to a maximum by the administration of androgen (Kochakian and Harrison, 1962; Kochakian, 1965). The RNA[1] of the nuclear, soluble, and mitochondrial fractions change essentially in direct proportion with the changes in weight of the kidney but the concentration of the microsomal RNA is decreased after castration and increased on androgen administration (Kochakian, 1965). Since orotic acid is a known specific precursor of the pyrimidines (Arvidson *et al.*, 1949; Hurlbert and Potter, 1952), the ^{14}C-labeled compound has been used to provide further information concerning the details of the mechanism of regulation of RNA synthesis in the mouse kidney by androgens.

Methods and Materials

Mice. The mice were from our colony (Kochakian and Harrison, 1962).

Materials. The steroid hormones[2] were implanted subcutaneously as cylindrical pellets (Kochakian, 1944). The orotic acid-6-^{14}C, 5.2 mc/mmole, was purchased from California Biochemical Corp.

Preparation of Homogenates. The food was removed 16 hr before autopsy. The kidneys were homogenized in cold 0.25 M sucrose (10% w/v) and fractionated by centrifugation (Hogeboom, 1955).

Extraction of Nucleic Acids. Aliquots in at least duplicate of the various cellular fractions were immediately extracted for nucleic acids with cold $HClO_4$ (Schneider, 1946; Volkin and Cohn, 1954).

Determination of Base Composition. The nucleic acids were extracted from the protein–nucleic acid powder with 10% NaCl (Hecht and Potter, 1956). The nucleotides of the alkali-hydrolyzed RNA were separated by ion-exchange chromatography (Katz and Comb, 1963).

Separation of Acid-Soluble Fraction. The acid-soluble fraction was immediately adsorbed and eluted from Norit A (Tsuboi and Price, 1959) and separated into the four groups of compounds (adenine = A, guanine = G, cytosine = C, uracil = U) (Katz and Comb, 1963) except that the U compounds were eluted with 0.01 N instead of 0.05 N HCl. The U group was fractionated by an adaptation of a procedure for sugars and their phosphates (Khym and Cohn, 1953). To the eluate (8 ml) was added 0.23 ml of 0.1 M $K_2B_4O_7$ and 0.68 ml of 1 N NH_4OH to give a pH of *ca.* 11. The solution was transferred to a chromatography column containing 0.9 × 5 cm of Dowex 1-X10 chloride pre-

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[1] Abbreviations used: RNA, ribonucleic acid; UMP, UDP, and UTP, uridine mono-, di-, and triphosphates; PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; DNA, deoxyribonucleic acid.

[2] The testosterone was purchased from G. D. Searle Co. and the androstan-17β-ol-3-one was provided by Syntex, S. A.

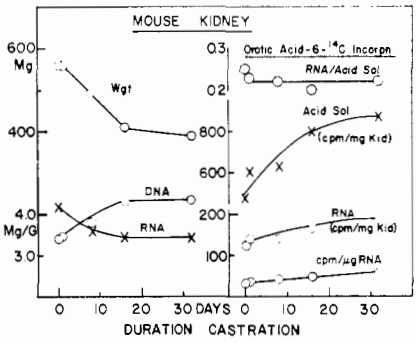

FIGURE 1: The effect of castration on the incorporation of orotic acid-6-^{14}C into the RNA and the acid-soluble fraction of the mouse kidney. The mice were castrated between 93 and 125 days of age so that the age of each group was the same at autopsy. There were five normal and three castrated mice per group. The food was removed at 5 P.M. and 3 µc of orotic acid-6-^{14}C was injected intraperitoneally 17–18 hr later; the mice were killed after 2 hr.

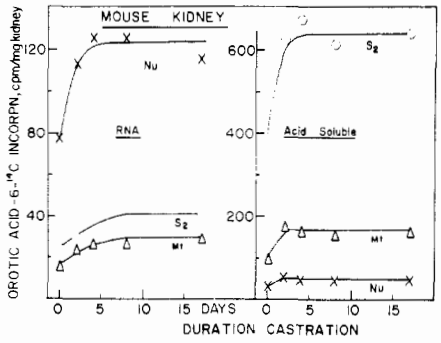

FIGURE 2: The effect of castration on the incorporation of orotic acid-6-^{14}C into the RNA and the acid-soluble materials of the particulate fractions of the kidney of the mouse. The mice were born at the same time and castrated at 168–170 days of age. There were ten normal and four to eight castrated mice per group. The kidneys of two mice were pooled for each analysis. The food was removed 17–18 hr before autopsy and 3 µc of orotic acid was injected intraperitoneally 2 hr prior to autopsy.

viously washed with 5 ml each of 1 N NH_4OH, 1 M NH_4Cl, 3 N HCl, and water until neutral to litmus. The flow rate was adjusted to 1 ml/min by air pressure. The uracil was eluted by 25 ml of a solution of 0.0025 N NH_4OH, 0.0005 M $K_2B_4O_7$, and 0.01 M NH_4Cl and the pH was adjusted to 7.8 with H_3BO_3. The uridine, UMP, UDP, and UTP were eluted successively by 15 ml each of 0.05 M, 0.15 M, 0.23 M, and 0.40 M NH_4Cl. The nucleotide content of the eluates was determined by their respective extinction coefficients (Dawson *et al.*, 1959).

Nucleic Acid Determinations. The total nucleic acids were determined from the absorbancy of the hydrolysates at 262 mµ (DeDeken-Grensen and DeDeken, 1959). The DNA was determined colorimetrically (Burton, 1956). The RNA was determined by subtraction of the DNA value from the total nucleic acid value.

Determination of Radioactivity. The radioactivity was determined in either a Packard Tri-Carb[3] Model 500A or a Nuclear Chicago Model[3] 724 apparatus. The sample, 1 ml, was added to 15 ml of a scintillation mixture containing 150 g of naphthalene, 7 g of PPO (2,5-diphenyloxazole), 100 mg of POPOP (1,4-bis[2-(5-phenyloxazolyl)]benzene), 100 ml of methanol (absolute), 20 ml of ethylene glycol, and spectroquality dioxane to make 1000 ml.

Results

Duration of Castration. The weight of the kidney and the concentration of RNA decreased and the concentration of DNA increased (Figure 1) as expected (Kochakian and Harrison, 1962). The radioactivity of the RNA increased immediately after castration and was accompanied by a proportionate increase in the radioactivity in the acid-soluble fraction. Each particulate fraction (Figure 2) showed changes similar to that of the total homogenate (Figure 1).

Time Course of Orotic Acid Incorporation. The greater incorporation of radioactivity in the RNA of the kidney of the castrated mouse became most apparent between 30 and 120 min after the injection of the orotic acid (Figure 3) and was evident in each particulate fraction. The total homogenate gave results comparable to the soluble fraction and was not plotted.

The radioactivity of the acid-soluble fraction was also higher in the castrated mice (Figure 4). The increase in the soluble (and homogenate) fraction was evident as early as 10 min after injection of the orotic acid. The administration of testosterone propionate for 2 days was ineffective.

Effect of Testosterone Propionate. The incorporation of radioactivity in the RNA of the total homogenate and the various particulate fractions was reduced to the normal level within 7 days of androgen treatment (Figure 5). Extension of the androgen administration to 14 and 21 days maintained the rate of incorporation at the normal level. The radioactivity in the acid-soluble material of the various subcellular fractions decreased to the normal level (Figure 6) simultaneously with that of the RNA. Androstan-17β-ol-3-one administration for 7 and 21 days produced results essentially identical with those produced by testosterone propionate. The data are not presented.

Radioactivity in the RNA Nucleotides. The incorporation of orotic acid in the mouse kidney was similar to that in the rat liver (Arvidson *et al.*, 1949; Hurlbert and Potter, 1952). No radioactivity was found in the DNA

[3] These instruments were kindly made available by Dr. R. Hanson, Pharmacology Department, and Dr. C. Pittman, Department of Medicine.

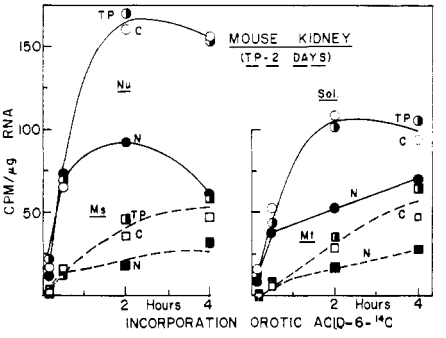

FIGURE 3: Effect of castration and testosterone propionate on the rate of incorporation of orotic acid-6-^{14}C into the RNA of the various fractions of the mouse kidney. The mice were all born at the same time. Castration was performed at 28–30 days of age (17–21 g of body weight). The testosterone propionate was implanted subcutaneously as a cylindrical pellet 2 days prior to autopsy; the average amount absorbed was 0.52 mg. The mice were 248, 269, 235, and 291 days of age for the 10 min, 30 min, 2 hr, and 4 hr experiments, respectively. Food was removed at 5 P.M. of the previous day and the 3 μc of orotic acid-6-^{14}C was injected intraperitoneally between 8 and 9 A.M. of the following morning. There were five mice per group, N = normal, C = castrated, TP = castrated plus testosterone propionate for 2 days. Nu = nuclear, Ms = microsomal, Sol = soluble, and Mt = mitochondrial fractions.

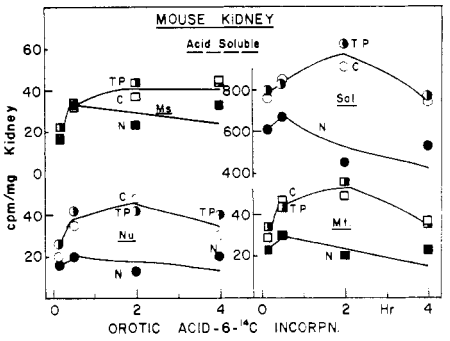

FIGURE 4: The effect of castration and testosterone propionate on the rate of incorporation of orotic acid-6-^{14}C into the acid-soluble material of the mouse kidney. See Figure 3 for experimental details. The values for the total homogenates gave the same pattern of response as the soluble fraction and have been omitted. Note that the soluble fraction contains most of the radioactivity.

and the trace amounts, *ca.* 1%, in the AMP and GMP fractions of the RNA nucleotides were due to slight contamination with CMP and UMP, respectively. The rest of the radioactivity was completely recovered in the two pyrimidines.

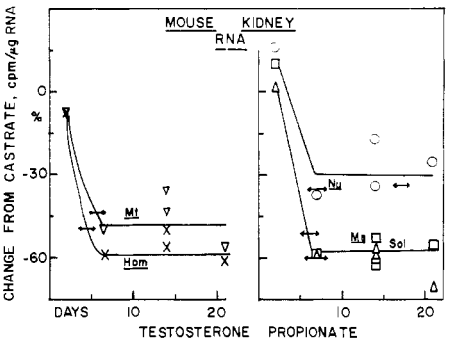

FIGURE 5: Effect of testosterone propionate on the incorporation of orotic acid-6-^{14}C into the RNA of the particulate fractions of kidneys from castrated mice. The mice were castrated at 28–30 days of age. The testosterone propionate was implanted subcutaneously as a cylindrical pellet at the following ages: 235 days for the 2-day experiment, 62 and 97 days for the two experiments at 7 days, 153 days for the 14-day experiment, and 76 days for the 21-day experiment. There were five mice per group except the second 7-day experiment had 7 normal, 12 castrated, and 10 testosterone propionate treated mice. The testosterone propionate absorbed was 0.52, 1.1, 2.1, and 3.4 mg for the respective periods. The double arrows indicate the level of the respective normal values.

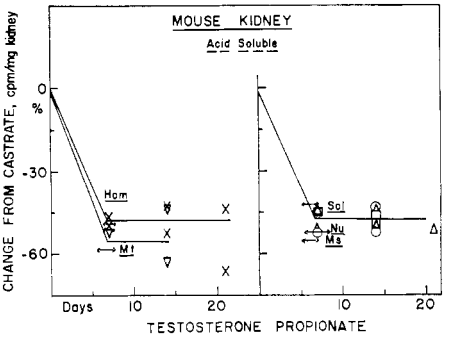

FIGURE 6: Effect of testosterone propionate on the incorporation of orotic acid-6-^{14}C into the acid-soluble material of the particulate fractions of mouse kidney. See Figure 5 for experimental details.

The incorporation of radioactivity was greater in the UMP than the CMP and was immediately increased by castration in both of the nucleotides (Figure 7). The effect of castration was not evident until 30 min after injection of the orotic acid (Figure 8). Furthermore, the rate of incorporation of radioactivity in the UMP of the nuclear fraction was not significantly altered by castration. The results in the mice treated for 2 days with testosterone propionate were identical with those of the castrated mice (Figure 8). The administration of testosterone propionate for 7 days, on the other hand, completely reversed the effect of castration (Table I).

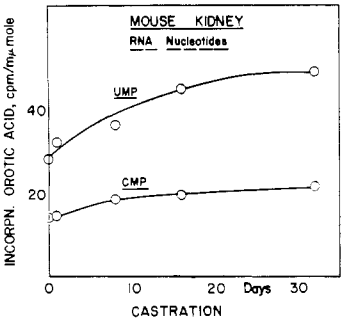

FIGURE 7: Effect of castration on the incorporation of orotic acid-6-^{14}C into the nucleotides of the RNA of mouse kidney. See Figure 1 for experimental details. The AMP and GMP fractions each contained only *ca.* 1% of the total radioactivity.

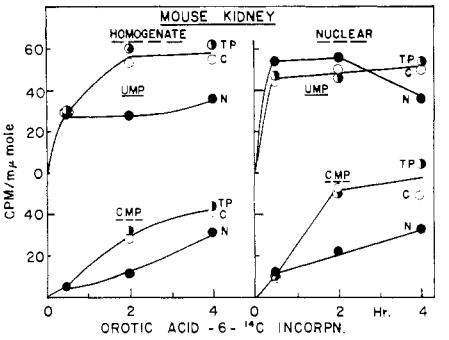

FIGURE 8: The effect of castration on the time course of incorporation of orotic acid-6-^{14}C into the RNA pyrimidines. See Figures 3 and 4 for experimental details. Note the lack of difference at 30 min.

TABLE I: The Effect of Castration and Testosterone Propionate for 7 Days on the Incorporation of Orotic Acid-6-^{14}C into the Nucleotides of the RNA of Mouse Kidney.[a]

Nucleotide	Normal	Castrate	Testosterone Propionate
	(cpm/mμmole)		
UMP	24.5	45.5	20.5
CMP	11.0	23.0	9.0
UMP/CMP	2.23	1.94	2.28

[a] See Figures 5 and 6 for experimental details. There were 7 normal, 12 castrated, and 10 testosterone propionate (TP) treated mice. The mice were 97 days of age. They were born at the same time (within 4 days). Castration was performed at 28–30 days of age. The testosterone propionate was implanted subcutaneously as a pellet 7 days before autopsy. The average kidney weights were: normal (N) 584 mg, castrated (C) 352 mg, and TP 508 mg. The food was removed 19–20 hr before autopsy and 3 μc of orotic acid-6-^{14}C was injected intraperitoneally 2 hr before autopsy. The AMP and GMP fractions contained only *ca.* 1% of the total radioactivity, the rest was recovered in the pyrimidines. No significant difference in the base composition of the RNA of the various groups was detected.

TABLE II: The Effect of Castration and Testosterone Propionate on the Concentration of Pyrimidine and Purine Components of the Acid-Soluble Fraction of Mouse Kidney.[a]

Components	Normal	Castrated	Testosterone Propionate
	(mμmoles/g of kidney)		
Uracil	252 ± 3.5	250 ± 19.0	223 ± 14.5
Uridine	597 ± 32.0	472 ± 9.0	343 ± 6.5
UMP	165 ± 9.5	201 ± 3.0	372 ± 0.0
UDP complex	243 ± 7.0	301 ± 9.0	454 ± 0.0
UTP	600 ± 14.0	705 ± 1.0	995 ± 9.5
Sum	1857	1929	2387
C	139 ± 3.0	156 ± 1.0	159 ± 2.5
G	241 ± 10.5	243 ± 8.0	361 ± 5.0
A	377 ± 3.5	374 ± 6.0	566 ± 1.0

[a] See Table I and Figures 5 and 6 for experimental details. The acid-soluble fractions were immediately pooled in duplicates representing 1160 mg of kidney/sample, adsorbed on Norit A, eluted, and analyzed. The values are the averages of duplicates with their differences. The C (cytidine), G (guanosine), and A (adenosine) complexes were not fractionated. Furthermore, the G fraction did not give the characteristic spectrophotometric absorption curve. The material was not further identified. The UDP fraction also contained related known and unknown components (Hurlbert *et al.*, 1954) but were not separated.

Composition of Acid-Soluble Fraction. The U compounds composed *ca.* 70% of the material in the acid-soluble fraction (Table II). Castration had no effect on the various components. The small changes in the uridine and the nucleotides were not supported by another study in which the greatest differences between castrated and normal mice were +8 and −15%. The administration of testosterone propionate for 7 days had absolutely no effect on the concentration of uracil, decreased that of uridine, and increased the concentration of the nucleotides. The concentration of all of the U compounds was practically the same for the normal and castrated mice but was increased in the androgen-treated castrated mice. Castration had no effect on the concentration of the G, C, or A groups.

The androgen, however, increased that of the G and A groups but had no effect on the C group.

Radioactivity in the Pyrimidines and Purines of the Acid-Soluble Fraction. Castration increased almost twofold the specific radioactivity of all of the components except that of the A group which had only an insignificant trace (0.3%) of radioactivity (Table III). The radioactivity in the G group also was small, *ca.* 2.5%, and could represent incompletely separated material from the U group. An approximately twofold increase in the specific radioactivities also has been observed after castration in not only fasted but also fed mice (C. D. Kochakian, unpublished). The administration of testosterone propionate restored the specific activity of each component to essentially the normal level except for uracil and UTP. The specific radioactivity of the uracil was decreased (−32%) to below the normal while that of the UTP remained at the increased level (+110%).

TABLE III: The Effect of Castration and Testosterone Propionate on the Specific Radioactivity of the Purine and Pyrimidine Compounds of the Acid-Soluble Fraction of the Kidneys of Mice Injected with Orotic Acid-6-^{14}C.[a]

Components	Normal	Castrated	Testosterone Propionate
	(cpm/mμmole)		
Uracil	284 ± 13.0	546 ± 41.0	193 ± 15.0
Uridine	219 ± 18.5	659 ± 20.0	277 ± 3.5
UMP	266 ± 20.0	629 ± 1.0	280 ± 1.0
UDP complex	92 ± 5.5	191 ± 1.0	119 ± 0.5
UTP	9 ± 0.0	16 ± 0.0	19 ± 0.5
C	112 ± 5.5	232 ± 0.5	108 ± 2.0
G	38 ± 9.5	71 ± 0.5	24 ± 1.0
A	0.4 ± 0.10	0.6 ± 0.10	0.1 ± 0.0

[a] See Tables I and II and Figures 5 and 6 for experimental details. The trace amount of radioactivity in the A group and the small amount in the G group are very probably due to the slight incomplete separation from the C and U groups, respectively. Indeed, the slight radioactivity in especially the A compounds attests to the effectiveness of the separation of the groups of compounds.

Discussion

The increase in specific radioactivity of the RNA of the kidney of the mouse after castration appeared at first to be anomalous since the increase was accompanied by a decrease of RNA. Analysis of the acid-soluble fraction, however, provided an explanation. The concentration of the RNA precursors and related substances was not significantly altered by castration. Thus, the level of the endogenous pool was maintained. The specific radioactivity of all of these components, however, was increased to the same degree as that of the RNA and its pyrimidines. It would seem, therefore, that the removal of the androgen resulted in a greater metabolism of the RNA precursors in both the anabolic and catabolic phases. Consequently, the RNA polymerase systems for the various subcellular fractions of the cell were provided with precursors of higher specific radioactivity.

It is of interest to note that the difference between the normal and castrated mice was apparent earlier in the acid-soluble fraction than in the RNA. The greater specific radioactivity of the RNA in the castrated mice occurred sometime between 30 and 120 min after administration of the tracer dose of orotic acid-6-^{14}C. It was not apparent after 10 and 30 min. On the other hand, the greater specific radioactivity in the acid-soluble fraction of the castrated mice was evident within 10 min after injection of the orotic acid-6-^{14}C. The increase occurred not only in the total homogenate and soluble fraction but also in the subcellular fractions except for the microsomal fraction which did not show a difference until 2 hr after the injection of the orotic acid. The practically identical pattern of response of the acid-soluble fraction of the different particulate fractions with that of the soluble cellular fraction suggests that the acid-soluble precursors are freely diffusible through the membranes of the nuclei, mitochondria, and endoplasmic reticulum as well as the cell. On the other hand, the presence of acid-soluble material in these subcellular fractions could represent residual soluble material. The amount of radioactivity present in all of these fractions was <10% of the total.

The administration of androgen increased the RNA of the kidney but decreased the specific radioactivity to the normal level with a concomitant decrease in the specific radioactivity of the acid-soluble fraction. Fractionation of the acid-soluble fraction indicated that the androgen decreased the specific radioactivity of uracil to below normal and that of UTP was maintained at the elevated level while the other U components and the C group were restored to the normal level.

The results with UTP suggest further study. This component was present in the highest concentration of not only the respective U compounds but it was also greater than each of the other groups. The radioactivity incorporated in the UTP, on the other hand, was the lowest. The nucleotide triphosphates are presumed to be the immediate precursors for the synthesis of RNA by the polymerases (Weiss, 1960). Actually the specific radioactivities of the UMP from the RNA of the normal and castrated mice were approximately three times that of its presumed precursor UTP while that of the testosterone propionate treated mice was approximately the same. The disparity, however, may not be real. The acid-soluble fraction represents the sum of a large number of compartments or pools in which the rate of synthesis and turnover of the various nucleotides could vary extensively (Petrovic *et al.*, 1964; Canellakis, 1957).

Furthermore, the nucleotides are not produced exclusively as precursors for RNA synthesis but are utilized in many metabolic processes.

Fractionation of the cytosine, guanosine, and adenosine groups into their separate compounds would be of interest but would require larger amounts of tissue even with the micro procedure employed because of the much smaller quantities of these compounds. It would be specially valuable to know the distribution of the radioactivity in the cytosine group and the distribution of the increase in the adenosine group.

The greater metabolism of the RNA in the castrated mice may be due to a decrease in protein synthesis for the formation of ribonucleoproteins and membranes for attachment of the RNA. Castration produces a sharp decrease in the biosynthesis of protein by the mouse kidney (Kochakian *et al.*, 1963; Kochakian, 1965) and also a decrease in the endoplasmic reticulum (Failoni and Scarpelli, 1965), the size of the mitochondria (C. D. Kochakian, unpublished), and the nuclear volume (Rabinovitch and Valeri, 1952; Bern and Alfert, 1954). Furthermore, the RNA polymerases decrease (J. Dubovsky and C. D. Kochakian, unpublished).

In a few of the experiments the seminal vesicles and prostates also were analyzed. The specific radioactivities of the RNA and the acid-soluble fraction were greater in the castrated than in the normal mice. Thus, the metabolism of the RNA in these tissues seemed to follow the same pattern as that in the kidney after castration.

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