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Biosynthesis of the Major Urinary Proteins in Mouse Liver: A Biochemical Genetic Study

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By labeling liver protein in vivo *with [^{3}H]leucine, the relative biosynthetic rate has been measured for the major urinary proteins (MUPs), three closely related, androgen-regulated proteins that are synthesized in mouse liver, secreted into the bloodstream, and excreted into the urine. In livers from females of strain C57BL/6J, total MUP synthesis represents about 0.6–0.9% of the total protein synthesis; in males and testosterone-treated females of the same strain, synthesis increases to about 3.5–4.0% of the total. This 4- to 6-fold induction of total MUP synthesis is similar to the androgen-mediated increase in MUP-specific messenger RNA reported by others, and indicates that the previously observed 20- to 25-fold induction of total MUP excretion into urine is generated partly at the posttranslational level. By measuring the ratio of synthesis of the individual MUPs, it was determined that the testosterone-mediated change in the relative levels of the MUPs in urine reflects a similar change in the pattern of MUP synthesis, indicating that the posttranslational processes operate on the quantity, and not the nature, of MUPs excreted. A survey of seven inbred mouse strains revealed polymorphism for the rate of total MUP synthesis in untreated females. Two classes could be distinguished on the basis of a 3- to 5-fold difference in the rate. This variation does not correlate with variation at* Mup-a, *a locus that controls the ratio of the three MUPs in urine from androgen-induced mice. These findings are consistent with the notion that MUP expression is controlled by a variety of independently assorting genes.*

KEY WORDS: major urinary proteins; rate of synthesis; androgen regulation; mouse liver.

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INTRODUCTION

A significant amount of protein synthesis in murine liver is devoted to the production of the major urinary proteins (MUPs), a family of three electrophoretically distinct, yet antigenically similar proteins that are rapidly secreted into the bloodstream and excreted into the urine (Finlayson *et al.*, 1963, 1965, 1974; Hudson *et al.*, 1967). Both the quantity and the nature of the MUPs in mouse urine are regulated by androgens. Males and testosterone-treated females excrete 20- to 25-fold more MUP than untreated females (Szoka and Paigen, 1978). In addition, in most strains, the relative ratio of the three MUPs excreted into the urine of males or testosterone-treated females is very different from that excreted into the urine of untreated females (Finlayson *et al.*, 1963; Szoka and Paigen, 1978). A regulatory locus, *Mup-a* on chromosome 4, has been shown to control the relative levels of the three MUPs in the urine from testosterone-induced animals (Hudson *et al.*, 1967; Szoka and Paigen, 1978). The relative levels in urine from untreated females are independent of *Mup-a* (Szoka and Paigen, 1979).

In vitro translation of liver messenger RNA (mRNA) using a wheat germ (Osawa and Tomino, 1977) or a mammalian fractionated cell-free (Szoka *et al.*, 1980) system has demonstrated that the level of translatable MUP mRNA is induced by testosterone. Furthermore, hybridization studies with a nucleic acid probe specific for MUP mRNA sequences have also shown that testosterone increases the level of total MUP mRNA (Hastie *et al.*, 1979). A peculiar finding in these studies was that MUP mRNA levels, as determined by the *in vitro* translation or the hybridization assay, increased about 4- to 6-fold in response to testosterone (Osawa and Tomino, 1977; Hastie *et al.*, 1979); this is in marked contrast to the 20- to 25-fold increase in the rate of MUP excretion into the urine (Szoka and Paigen, 1978). This discrepancy between induction of the MUP mRNA level and of MUP excretion could be due to either of two factors. MUP synthesis may be regulated at the translational level, i.e., the efficiency of translation of MUP mRNA *in vivo* following testosterone induction may increase relative to the efficiency of translation in the absence of androgen; thus a 4- to 6-fold increase in the MUP mRNA level may result in an even greater increase in the rate of MUP synthesis, leading to a greater than 4- to 6-fold increase in the MUP level in urine. Alternatively, the 4- to 6-fold induction of the MUP mRNA level may be paralleled by an equivalent induction of the rate of MUP synthesis; the large increase in the urinary MUP level would then be due to some posttranslational process that is affected by testosterone and that is involved in some aspect of MUP excretion.

In this paper, these possibilities have been distinguished by direct measurement of the rate of total MUP synthesis *in vivo*. This rate, like the

concentration of MUP mRNA, was found to be induced 4- to 6-fold by testosterone, indicating that the large androgen-dependent increase in urinary MUP levels is generated partly at the posttranslational level. In addition, it was determined that the relative levels of the three MUPs in urine from untreated and testosterone-treated mice reflects the pattern of MUP synthesis. Finally, genetic variations in the rate of MUP synthesis and its inducibility among inbred mouse strains have been identified. Among those strains tested, the variations are not correlated with the distribution of *Mup-a* alleles; hence, the locus or loci controlling the synthesis of MUP are probably distinct from *Mup-a,* attesting to the complex genetic nature of MUP regulation (Szoka and Paigen, 1978, 1979).

MATERIALS AND METHODS

Animals

All inbred mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. They were used at 2 months of age.

Purification of MUP and Preparation of Antibody

The three MUPs were purified from the urine of testosterone-treated female mice of strain C57BL/6J by Sephadex G-100 chromatography followed by DEAE-cellulose chromatography (Szoka and Paigen, 1978). A mixture of the three purified MUPs was used to raise antibody in the goat. The IgG fraction was isolated from serum containing a high titer of anti-MUP activity by precipitation twice in 40% ammonium sulfate and dissolving in 0.02 M Tris–Cl, *p*H 7.5, containing 0.15 M sodium chloride.

Induction

Female mice were induced with androgen by the subcutaneous application of 30-mg testosterone pellets for 3 weeks, unless otherwise stated. The pellets were obtained from Dr. Bhogi B. Sheth of the Department of Pharmaceutics at the University of Tennessee.

Measurement of the Rate of Total MUP Synthesis

Animals were injected intraperitoneally with 100 μCi L-[4,5-^{3}H]leucine (40–60 Ci/mmol; Amersham) and sacrificed 5–7 min later by cervical

dislocation. Livers were rapidly removed, chilled, and homogenized in 5 vol (w/v) of 0.1 M Tris–Cl, *p*H 7.5, containing 0.15 M sodium chloride using a Polytron homogenizer. Radioactivity incorporated into total protein was determined by precipitation of a 10-μl aliquot of the homogenate with 5% trichloroacetic acid (Berger *et al.*, 1978). Homogenates were brought to 1% Triton X-100 and 0.5% sodium deoxycholate and centrifuged at 10,000 rpm for 30 min; the clear supernatant between the pellet and the floating lipid layer was removed. Mixtures for antibody precipitation contained, in a total volume of 0.5 ml, the following: 0.02 M Tris–Cl, *p*H 7.5, 0.15 M sodium chloride, 0.2 M L-leucine, 0.5% sodium deoxycholate, 1.0% Triton X-100, 1% bovine serum albumin, 25 μg of purified MUP, an appropriate volume of anti-MUP IgG, and liver supernatant (usually 100–200 μl prepared as described above). The mixtures were incubated for 30 minutes at 37 C, then overnight at 4 C. They were centrifuged at 5000 rpm for 15 min through a cushion of 0.02 M Tris–Cl, *p*H 7.5, containing 1.0% Triton X-100, 0.5% sodium deoxycholate, and 1.0 M sucrose. The pelleted antibody–antigen complexes were washed, dissolved, and electrophoresed through polyacrylamide disc gels containing 12.5% acrylamide and 0.2% *N,N'*-dimethylenebisacrylamide in the presence of sodium dodecyl sulfate (Berger *et al.*, 1978). Gels were stained for protein using 0.25% Coomassie brilliant blue R-250 in methanol:acetic acid:H_2O (5:1:5), then destained in methanol:acetic acid:H_2O (5:1:5). The gels were cut into 2-mm slices, and the radioactivity was determined in each slice as previously described (Berger *et al.*, 1978). The relative rate of total MUP synthesis is expressed as the ratio of radioactivity incorporated into MUP to that incorporated into total liver protein.

Measurement of Synthesis of the Individual MUPs

The synthesis of each MUP was measured following separation by isoelectric focusing in acrylamide gels. Immunoprecipitates, prepared as described above, were dissolved in 9.5 M urea, 2% (w/v) Nonidet P-40, 5% β-mercaptoethanol, and 0.05% sodium dodecyl sulfate. Samples were applied to isoelectric focusing disc gels, containing 9 M urea, 4% acrylamide, 0.2% *N,N'*-dimethylenebisacrylamide, 2% (w/v) Nonidet P-40, 1.6% ampholines, *p*H 4–6 (LKB), 0.4% ampholines, *p*H 3.5–10 (LKB), 0.01% ammonium persulfate, and 0.07% (v/v) TEMED. The anode buffer was 0.01 M H_3PO_4, while the cathode buffer was 0.02 M NaOH. Focusing was at 200 V for 2 hr, 400 V for 12–16 hr, and 800 V for 1 hr. Gels were cut into 1-mm slices, and radioactivity was extracted and measured in each slice (Berger *et al.*, 1978).

RESULTS

Determination of the Relative Rate of Total MUP Synthesis

The *in vivo* rate of total MUP synthesis was determined by measuring the incorporation of radioactivity into the MUPs in the liver of an animal injected with a labeled amino acid. Because the MUPs are rapidly secreted from the liver and excreted into the urine (Finlayson *et al.*, 1965), a labeling time was chosen that was short enough to prevent any newly synthesized MUP from escaping into the bloodstream and causing inaccurate rate measurements. As a preliminary experiment, [^{3}H]leucine was injected into each of several mice, and the appearance of leucine-labeled plasma protein was determined. Figure 1 shows that there is an insignificant amount of labeled plasma protein up to 10 min postinjection; subsequently, there is an abrupt increase in the labeling that continues for at least an hour. Similar observations have been made in the rat (Schreiber *et al.*, 1969). Thus, for measurement of total MUP synthesis, a labeling time of less than 10 min was chosen.

Male mice of strain C57BL/6J were injected with [^{3}H]-leucine; after 5–7 min, the livers were rapidly removed and homogenized. The MUPs were quantitatively precipitated with antibody and the immunoprecipitates were solubilized and electrophoresed through polyacrylamide disc gels in the presence of sodium dodecyl sulfate. Staining the gels for protein revealed three prevalent bands: a heterogeneous band covering a molecular weight range of 50,000–60,000 represents heavy antibody chains; another heterogeneous band covers a molecular weight range of 25,000–30,000 and represents light antibody chains; the third band, MUP, migrates as a species having molecular weight 19,000, which is very similar to that reported previously (Finlayson *et al.*, 1974; Szoka *et al.*, 1980). By slicing the gels and determining the radioactivity in each slice, it was found that 80–90% of the label comigrated with MUP (Fig. 2). The relative rate of total MUP

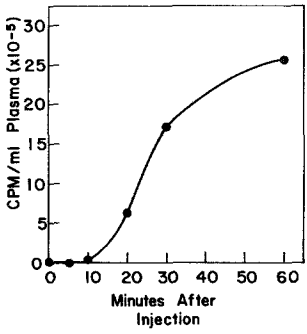

Fig. 1. Labeling of plasma proteins with [^{3}H]-leucine *in vivo*. Mice of strain C57BL/6J were injected intraperitoneally with 100 μCi of L-[4,5-^{3}H]leucine. For each time point, two mice were sacrificed, the portal veins were severed, and blood samples were collected in heparinized tubes. The samples were centrifuged at 10,000 rpm for 30 min, and radioactivity in total protein in the plasma supernatant was determined by precipitation with trichloroacetic acid.

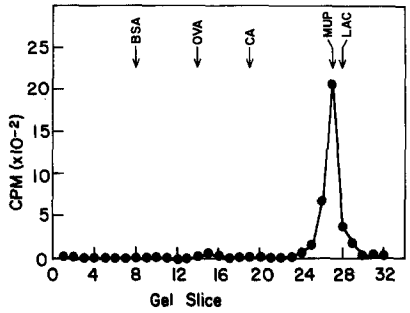

Fig. 2. Profile of radioactivity following polyacrylamide gel electrophoresis of MUP immunoprecipitates in the presence of sodium dodecyl sulfate. MUP immunoprecipitates, prepared from male mice of strain C57BL/6J as described in Materials and Methods, were washed, solubilized, and electrophoresed in 12.5% acrylamide disc gels in the presence of sodium dodecyl sulfate (Berger *et al.*, 1978). Following electrophoresis, the gels were stained for protein with Coomassie brilliant blue; they were sliced, and slices were extracted and counted. Molecular weight standards: BSA (bovine serum albumin; molecular weight = 68,000); OVA (ovalbumin; molecular weight = 45,000); CA (carbonic anhydrase; molecular weight = 32,000); MUP (authentic MUP; molecular weight = 19,000); and LAC (β-lactalbumin; molecular weight = 18,000).

synthesis, expressed as the ratio of radioactivity incorported into the MUP peak to that incorporated into total homogenate protein, was found to be 0.035 in the experiment depicted in Fig. 2. The range of values for several determinations was 0.033–0.041. Similar measurements by others for α_{2u}-globulin, the analogous urinary protein in male rats, revealed a slightly lower value of 0.009–0.010 (Sippel *et al.*, 1976).

Effect of Testosterone on the Relative Rate of MUP Synthesis

The relative rate of total MUP synthesis was measured both before and after induction with androgen. Female mice of strain C57BL/6J were treated with

Table I. Effect of Testosterone on the Relative Rate of MUP Synthesis[a]

Treatment	Total protein synthesis (cpm/g liver)	Total MUP synthesis (cpm/g liver)	Rate of MUP synthesis
None	4,650,000	43,100	0.0093
Testosterone	5,160,000	207,800	0.040

[a]Female mice of strain C57BL/6J were treated with testosterone, and the rate of MUP synthesis in liver was measured following injection with [^{3}H]-leucine as described in Materials and Methods. Values were obtained by pooling the livers of three animals and averaging duplicate measurements of each pool.

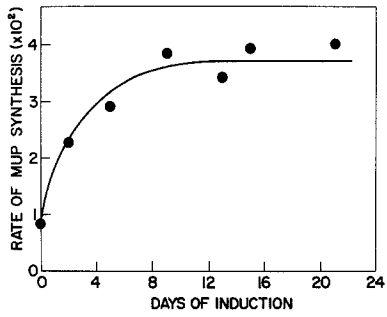

Fig. 3. Kinetics of androgen induction of MUP biosynthesis. Female mice of strain C57BL/6J were induced on day 0 with testosterone pellets. At various times, rates of total MUP synthesis were determined as described in Materials and Methods. Each point represents the average of duplicate measurements on a pool of two livers.

testosterone for 21 days, after which the relative rate of total MUP synthesis was measured and compared to that in untreated females. A typical result is shown in Table I. There was no significant difference in total protein synthesis between androgen-treated and control animals; however, the relative synthesis of the MUPs increased almost 5-fold, from about 0.0093 to 0.040. This correlates well with the testosterone-mediated increase in MUP mRNA levels reported previously (Osawa and Tomino, 1977; Hastie *et al.*, 1979) and suggests that part of the 20- to 25-fold increase in urinary MUP must be generated posttranslationally.

The kinetics of induction were studied by measuring total MUP synthesis over the 21-day period following testosterone implantation (Fig. 3). Full induction of MUP synthesis takes about 7–8 days and remains at that level for at least 2 more weeks. Recent experiments (P. R. Szoka, unpublished) show that the MUP mRNA activities, as measured in cell-free reticulocyte lysates, parallel the rates of synthesis throughout the induction period.

Measurement of the Synthesis of the Individual MUPs

Previous work has shown that the relative levels of the three MUPs in the urine are changed dramatically by testosterone treatment (Finlayson *et al.*, 1963; Szoka and Paigen, 1978). For example, in C57BL/6J females, the ratio of MUP-1:MUP-2:MUP-3 changes from 0:5:1 to 1:2:2. It was shown above that posttranslational events participate in determining the quantity of total MUPs excreted into urine; it is conceivable that the change in the urinary MUP ratio upon androgen treatment is also generated posttranslationally. Therefore the ratio of synthesis of the individual MUPs was examined.

Following *in vivo* labeling of C57BL/6J mice and preparation of an immunoprecipitate in the usual fashion, the radioactive MUPs were separated by isoelectric focusing in acylamide gels. The patterns of radioactivity in resulting gel slices are shown in Fig. 4. Testosterone-induced mice (Fig. 4A) show four major peaks of radioactivity (labeled I–IV); the ratio of peaks

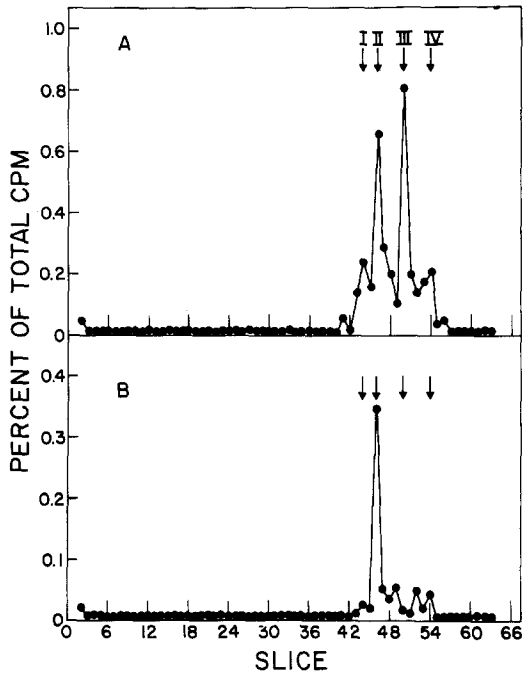

Fig. 4. Profile of radioactivity following isoelectric focusing of MUP immunoprecipitates. MUP immunoprecipitates, prepared from testosterone-induced (A) or basal (B) C57BL/6J mice, were solubilized and applied to isoelectric focusing gels. Following electrophoresis, gels were sliced, extracted, and counted. The percentage of the total liver homogenate cpm was plotted as a function of the slice number, starting at the cathodal end of the gel. The arrows mark the four prominent peaks, labeled I–IV, in the profile from testosterone-induced mice.

I:II:III correlates fairly well with the reported ratio of MUP-1:MUP-2:MUP-3 in urine from androgen-induced mice (Szoka and Paigen, 1978). Untreated females (Fig. 4B) show a quite different pattern. A single peak of radioactivity, corresponding to peak II in the induced profile, predominates; this pattern reflects what is found in female urine, where >80% of the excreted MUP is MUP-2 (Szoka and Paigen, 1978).

There appear to be minor, though significant, levels of other radioactive species. These are probably additional MUPs, since the total amounts of radioactivity recovered from the isoelectric focusing gels were nearly identical to the amounts which comigrate with the MUPs on denaturing acrylamide gels. However, the possibility that these extra species are unrelated contaminants cannot be definitively ruled out.

Genetic Variation in the Relative Rate of MUP Synthesis

Earlier studies identified a genetic locus, *Mup-a,* that controls the relative levels of the three MUPs in urine following androgen induction (Hudson *et al.,* 1967; Szoka and Paigen, 1978). Since a large variation in the rate of total MUP excretion was observed among the mouse strains studied (Szoka and Paigen, 1978), it was of interest to ascertain whether this variation reflects polymorphism at the level of MUP biosynthesis. Therefore, the rate of total MUP synthesis in each of several inbred strains was measured, the results of which are summarized in Table II.

Basal rates of synthesis, i.e., in normal females, exhibit interstrain variation. Strains C57L/J, C3H/HeJ, AKR/J, and A/J have basal relative synthetic rates of 0.002–0.003 (Table II); these strains have generally low rates of MUP excretion (Szoka and Paigen, 1978). Strains C57BL/6J, C57BL/6ByJ, and DBA/2J, which excrete 2- to 5-fold greater quantities of MUP (Szoka and Paigen, 1978), have significantly high basal relative rates of total MUP synthesis (i.e., 0.006–0.009). Thus, the variations observed for the rates of MUP excretion are generally reflected by similar variations in the relative rate of MUP biosynthesis. However, genetic factors besides the rate of synthesis appear to play a role in determining MUP excretion; e.g., although strain C57BL/6ByJ excretes significantly more total MUP than either C57BL/6J or DBA/2J (Szoka and Paigen, 1978), its rate of total MUP synthesis is about the same as the latter two.

Interestingly, the variation for rate of total MUP synthesis is expressed only in normal females. When rates were measured in androgen-treated

Table II. Rate of Total MUP Synthesis in Inbred Mouse Strains[a]

Class	Inbred strain	*Mup-a* alele	Rate of total MUP synthesis ($\times 10^2$)		
			Basal	Induced	Fold induction
I	C57BL/6J	2	0.89 ± 0.08	4.2 ± 0.5	4.7
	C57BL/6ByJ	2	0.67 ± 0.07	3.3 ± 0.5	4.9
	DBA/2J	1	0.91 ± 0.23	4.8 ± 0.7	5.3
II	C3H/HeJ	1	0.27 ± 0.05	4.0 ± 0.9	14.8
	C57BL/J	2	0.28 ± 0.04	3.4 ± 0.4	12.0
	AKR/J	1	0.30 ± 0.07	4.2 ± 0.4	14.0
	A/J	1	0.21 ± 0.03	2.1 ± 0.3	10.0

[a]Three basal and three testosterone-treated females from each strain were injected with [^{3}H]-leucine and the basal and induced rates of MUP synthesis, respectively, were determined on individual livers as described in Materials and Methods. The *Mup-a* allele for each strain has been listed previously (Szoka and Paigen, 1978). Values for rates of synthesis represent the averages for the three determinations ± the standard deviations.

females, it was found all strains were nearly the same. The strains with high basal synthetic rates could be induced 4- to 6-fold (Table II), while the strains with low basal rates could be induced 10- to 15-fold (Table II). There is little, if any, correlation between induced levels of MUP synthesis and excretion in these strains, indicating that factors in addition to the biosynthetic rate for the MUPs determine induced excretion rates.

Thus, the basal rate of MUP synthesis and its inducibility by testosterone show variation among inbred mice that partially, though not completely, accounts for the variation in MUP excretion. It is pertinent to point out that these aspects of the MUP phenotype vary independently of *Mup-a* (Table II), suggesting that they are under control of separate genetic loci. The lack of strong correlation between variations in MUP synthesis and excretion reinforces the conclusion drawn from analysis of these parameters during testosterone induction, i.e., posttranslational events play a regulatory role in determination of urinary MUP levels.

DISCUSSION

Genetic and molecular analyses of MUP expression are revealing it to have great potential in addressing general questions on genetic regulation and the hormonal response in higher organisms. In this paper, the *in vivo* rate of MUP biosynthesis has been measured in order to gain insight into the relationships between MUP synthesis and excretion both before and after androgen induction. Such a study was prompted by the finding that testosterone increases the rate of MUP excretion into the urine by 20- to 25-fold (Szoka and Paigen, 1978) while inducing the MUP mRNA level only about 5-fold (Osawa and Tomino, 1977; Hastie *et al.*, 1979); in fact, MUP excretion in some strains is induced as much as 50-fold (Szoka and Paigen, 1978). Measurement of the rate of MUP synthesis enables determining whether or not this differential response to androgen is generated at the level of protein synthesis. Such a determination is of obvious importance in understanding the hormonal regulation of MUP expression.

Relative rates of synthesis were determined by measuring the incorporation of radioactivity into the MUPs a short time after the injection of a labeled amino acid precursor. The results (Table I) clearly indicate that in response to testosterone the relative rate of total MUP synthesis increases about 4- to 6-fold, which is comparable to the extent of MUP mRNA induction following hormone treatment (Osawa and Tomino, 1977; Hastie *et al.*, 1979). It appears that the large increase in the MUP excretion rate is not generated solely at the synthetic level, but involves some posttranslational event(s) that is androgen sensitive.

Although the degradation rate for the MUPs has not been measured, it

is unlikely that it is a factor in the rate of synthesis measurements. First, MUP accumulation in urine can be as great as 20 mg per mouse per day (Szoka and Paigen, 1978); if the intracellular degradation rate were great enough to interfere with the synthesis measurement in a 5- to 7-min pulse time, such accumulation would require a rate of MUP synthesis that almost exceeds the total capacity for protein synthesis in liver. Second, rate of synthesis measurements that have been made using a pulse time as long as 45 min are nearly identical to those made using a pulse time of 5–7 min (F. G. Berger, unpublished). Thus, the half-life for the MUPs in the liver is considerably longer than the pulse time for synthesis measurements.

The androgen-sensitive posttranslational process affecting the quantity of MUP excreted is not known; conceivably, any step between synthesis of the polypeptide chain and the appearance of it in the urine could be affected. Testosterone has large effects upon gene expression in kidney (Swank *et al.*, 1978); the hormone has also been shown to alter the fraction of lysosomal enzymes excreted from the proximal tubule cells of the kidney (Brandt *et al.*, 1975). Although the excretion pathway for the MUPs involves glomerular filtration, it is possible that MUP excretion and/or reabsorption by the kidney is affected by androgen.

Analysis of the rate of synthesis of individual MUPs (Fig. 4) shows that the testosterone-induced change in the MUP ratio in urine reflects a similar change in the pattern of MUP synthesis. Thus, the posttranslational events that participate in determination of total MUP levels in the urine do not appear to discriminate among the various MUPs, i.e., they act upon the quantity and not the nature of MUPs excreted. It has been suggested that the *Mup-a* locus controls the urinary MUP ratio in induced mice by regulating the relative concentration of mRNA for each MUP (Szoka *et al.*, 1980); regulation of the urinary MUP ratio by testosterone is probably accomplished in a similar fashion.

The heterogeneity of radioactive peaks observed in the isoelectric focusing gels in Fig. 4 suggests that there may be more MUPs than previously thought. This probably reflects the higher resolution of isoelectric focusing compared to conventional gel electrophoresis. That there are more than three MUPs has already been noted by others (Hudson *et al.*, 1967; Szoka and Paigen, 1978; Szoka *et al.*, 1980); in addition, recent protein chemical analyses of the MUPs indicate the existence of new charge and molecular weight forms (G. Homandberg, J. Knopf, and W. Held, personal communications). Further studies are needed before the exact nature of the MUP family is known.

The genetic regulation of MUP expression appears to be very complex, involving several independent genes which show extensive polymorphism among inbred strains. Variation at the *Mup-a* locus, mapped to chromosome

4, causes altered ratios of the three MUPs in induced urine (Hudson *et al.*, 1967; Szoka and Paigen, 1978). This altered ratio has recently been shown to be generated at the pretranslational level (Szoka *et al.*, 1979), suggesting that the MUPs are synthesized from separate mRNAs. Studies on the MUP ratios in uninduced animals indicate that genes in addition to *Mup-a* play a significant role in determining the ratios (Szoka and Paigen, 1978, 1979).

Measurement of the relative rates of total MUP synthesis in several strains allowed classifying them into two groups based upon variation in untreated females (see Table II). The rates do not correlate with the *Mup-a* alleles carried by the respective strains, an observation consistent with the notion that the genetic determinant(s) for rate of synthesis is unlinked to *Mup-a,* reinforcing the conclusion that various aspects of MUP expression are controlled by a variety of independently assorting genetic loci (Szoka and Paigen, 1979). More detailed examination of the genetics of MUP synthesis is currently underway to further establish and extend this conclusion.

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