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Studies on the Mode of Oestrogenic Inhibition of Hepatic Synthesis of α_{2u}-Globulin and its Corresponding Messenger Ribonucleic Acid in Rat Liver

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1. The possible mechanism of the oestrogenic inhibition of the androgen-dependent synthesis of α_{2u}-globulin in rat liver was explored by a correlative study of the amounts of α_{2u}-globulin, its corresponding mRNA and circulating testosterone in oestrogen-treated male rats. 2. Daily treatments of mature male rats with oestradiol-17β (10μg/100g body wt.) decreased and ultimately stopped the hepatic synthesis of α_{2u}-globulin as determined by both hepatic and urinary concentrations of the protein. The oestrogen-mediated decrease in the hepatic synthesis of α_{2u}-globulin was correlated with a decrease in the mRNA for this protein. 3. Withdrawal of oestrogen resulted in the recovery of α_{2u}-globulin synthesis and an increase in mRNA for α_{2u}-globulin. 4. At higher doses of oestradiol-17β (50μg/100g body wt.), synthesis of α_{2u}-globulin was totally suppressed. In addition, this treatment resulted in an extended period of androgen-insensitivity during which treatment with androgens induced synthesis of neither α_{2u}-globulin nor its corresponding mRNA. 5. It is concluded that the oestrogenic inhibition of α_{2u}-globulin synthesis is mediated by an oestrogen-dependent decrease in the hepatic content of translatable mRNA for α_{2u}-globulin.

In recent years, investigations with various model systems involving mammalian and avian reproductive tissues have led to considerable advancements in our understanding of the mechanism of action of sex hormones (see reviews by King & Mainwaring, 1974; O'Malley & Means, 1974). These studies indicate that the steroid hormones are first selectively concentrated by specific receptor proteins present in their target cells, and subsequent interaction between the steroid–receptor complex and the genetic apparatus leads to modulation of gene expression. Although regulation of gene transcription seems to be the primary mechanism for steroid-hormone action (Harris *et al.*, 1976), other possibilities such as post-transcriptional and post-translational regulation have not been completely ruled out (Tomkins *et al.*, 1969; Liang & Liao, 1975). We have developed a model system for studying the androgen-dependent synthesis of α_{2u}-globulin in rat liver (Roy & Neuhaus, 1967). This globulin is synthesized in the liver of male rats and is excreted in the urine (Roy *et al.*, 1966; Roy & Raber, 1972). Our system permits investigations of the regulation of a specific gene product in a non-reproductive tissue where sex hormones are not known to be involved in cell proliferation.

Several systems are now available for the quantitative assay of specific species of mRNA (Lockard & Lingrel, 1969; Mathews & Korner, 1970; Roberts & Paterson, 1973). It has been possible to translate and quantify the specific mRNA for α_{2u}-globulin in cell-free systems derived from wheat-germ and ascites-tumour cells (Sippel *et al.*, 1975; Roy *et al.*, 1976). Earlier investigations from our laboratory have shown that the androgenic induction of α_{2u}-globulin is associated with a simultaneous rise in the content of the mRNA for α_{2u}-globulin in rat liver (Sippel *et al.*, 1975). Because of the inhibitory effect of oestrogen on the synthesis of α_{2u}-globulin (Roy & Neuhaus, 1967; Roy *et al.*, 1975), we decided to investigate this inhibition in detail. A preliminary report on this topic has already been published (Dowbenko *et al.*, 1976).

Materials and Methods

Animals and treatments

Experiments were performed on albino rats of Yale strain (350–400g body wt.) obtained from Maguran Farms, Troy, MI, U.S.A. The animals were housed in an air-conditioned animal room and fed on Purina rat chow and tap water *ad libitum*. Oestradiol-17β and testosterone (Sigma Chemical Co., St. Louis, MO, U.S.A.) were administered subcutaneously as emulsions in 0.1M-sodium phosphate buffer (pH 7.2)/propylene glycol/Tween 80 (224:25:1, by vol.) as described by Kumar *et al.* (1969).

Processing of the urine, purification of α_{2u}-globulin and preparation of rabbit antiserum to α_{2u}-globulin

Urine samples were collected in stainless-steel metabolism cages with 0.5ml of a preservative

solution containing 1% (w/v) penicillin, 1% (w/v) streptomycin and thymol to saturation. Procedures for the purification of α_{2u}-globulin from male rat urine and preparation of rabbit antiserum to α_{2u} globulin have been described previously (Roy *et al.*, 1966; Roy, 1973).

Preparation of hepatic protein for the assay of α_{2u}-globulin

Liver samples were removed under ether anaesthesia and all subsequent operations were carried out at approx. 0°C. The liver was homogenized in 0.1M-Tris/HCl buffer, pH8.0 (10ml/g of liver), in a motor-driven Teflon/glass homogenizer with 15 up-and-down strokes. The homogenate was centrifuged at 27000*g* for 15min in a Sorvall refrigerated centrifuge (SS-34 rotor). The supernatant fraction below the top lipid layer was removed and was sonicated for 15s at setting 80 of a Bronwill Biosonik III sonicator (Bronwill Scientific, Rochester, NY, U.S.A.). The sonicated postmitochondrial supernatant was further centrifuged at 160000*g* for 120min in a Beckman L5-75 centrifuge (50 Ti rotor). The supernatant fraction obtained after the 160000*g* centrifugation was used for the determination of α_{2u}-globulin by radioimmunoassay. The total protein concentration of the supernatant was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Radioimmunoassay of α_{2u}-globulin

Urinary and hepatic contents of α_{2u}-globulin were determined by modification of the double-antibody radioimmunoassay procedure initially developed for the assay of pituitary hormones (Utiger *et al.*, 1962; Greenwood *et al.*, 1963). Carrier-free Na^{125}I obtained from Amersham/Searle Co., Des Plaines, IL, U.S.A. (11–17mCi/μg of I), was used in the iodination of α_{2u}-globulin with chloramine-T as the oxidizing agent. Radioiodinated α_{2u}-globulin was separated from the free ^{125}I by gel filtration through Sephadex G-50. Liver proteins and urine samples were diluted with phosphate/saline (0.01M-sodium phosphate buffer, pH7.6/0.15M-NaCl) and were used for the radioimmunoassay. A 0.1ml portion of the diluted sample was mixed with 0.1ml of ^{125}I-labelled α_{2u}-globulin, 0.2ml of diluted rabbit anti-(α_{2u}-globulin) serum and 0.6ml of albumin solution (1% bovine serum albumin in phosphate/saline). The mixture was incubated for 60min at 37°C, followed by 16h at 4°C. The complex was then precipitated by incubation with 0.2ml of goat anti-(rabbit γ-globulin). The mixture was incubated for 60min at 37°C and was centrifuged at 2000*g* for 15min. The supernatant containing labelled α_{2u}-globulin not bound to the antibody was removed, and the precipitate containing the antigen–antibody complex was washed with 0.5ml of the albumin solution and counted for radioactivity in a Searle automatic gamma counter. A standard curve was prepared with known concentrations of α_{2u}-globulin, and the unknown values were read from the standard curve. All assays were run in duplicate; the lowest limit of sensitivity for the assay was 2ng of α_{2u}-globulin/ml.

Radioimmunoassay of serum testosterone

Serum testosterone was determined by double-antibody radioimmunoassay. Testosterone radioimmunoassay kit containing rabbit antiserum against testosterone 3-*O*-carboxymethyloxime–bovine serum albumin, sheep antiserum to rabbit γ-globulin and ^{125}I-labelled testosterone 3-*O*-carboxymethyloxime–tyrosine methyl ester and standard testosterone were obtained from Serono Laboratories, Boston, MA, U.S.A. The assay procedure used was as described by the supplier. All assays were run in duplicate. The lower limit of sensitivity of the assay was 10pg/ml. According to the supplier, the anti-testosterone antiserum showed no cross-reaction with other steroids, except testosterone and 5α-dihydrotestosterone, even if present in greater-than-physiological amounts. 5α-Dihydrotestosterone cross-reacted with the antiserum to about 40% of the extent that testosterone did. Therefore, although the results are expressed as contents of serum testosterone, it may more accurately represent the content of serum androgen.

Extraction of hepatic mRNA and translation of the mRNA in vitro in wheat-germ cell-free systems

Total hepatic RNA was extracted with phenol and sodium dodecyl sulphate by the procedure of Rosenfeld *et al.* (1972). Poly(A)-containing RNA was obtained by chromatographic fractionation of the total RNA on oligo(dT)–cellulose as described by Aviv & Leder (1972). Wheat-germ S-30 fraction was prepared by the procedure of Roberts & Paterson (1973) as modified by Rosen *et al.* (1975): 11.0g of wheat-germ was ground at 0°C with 11.0g of acid-washed glass beads and 30ml of buffer, and the remaining procedure was as described by Rosen *et al.* (1975). The reaction mixture for the translation of the hepatic mRNA in the wheat-germ cell-free system was as follows: 20mM-Hepes* (pH7.6), 2mM-dithiothreitol, 1mM-ATP, 100μM-GTP, 60μM-CTP, 5mM-phosphocreatine, 12.8μg of creatine kinase (3.2mg/ml), 60mM-KCl, 1.6mM-magnesium acetate and 40μM each of 19 unlabelled amino acids. A 200μl reaction mixture contained 100μl of wheat-germ S-30 fraction, 60μg of poly(A)-containing RNA and 60μCi of [^{3}H]-leucine (59Ci/mmol). The mixture was incubated for 60min at 24°C. At the end of the incubation period, 5μl samples were spotted on Whatman no. 3 filter-paper discs (2.3cm diam.) and processed as described by

* Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Bollum (1968) for the determination of total protein synthesis. Filter discs were counted for radioactivity in a liquid-scintillation spectrometer (Packard) with 5ml of a scintillation 'cocktail' containing 0.5% Omnifluor (New England Nuclear Corp., Boston, MA, U.S.A.) in toluene. The rest of the reaction mixture was centrifuged at 160000*g* for 90min in a Beckman L5-75 ultracentrifuge (50 Ti rotor) to obtain the supernatant containing the released polypeptide chains. A sample (5μl) was also assayed by the filter-disc procedure for the measurement of total leucine incorporation into the released polypeptide chains. Counting efficiency of [^{3}H]leucine in minced polyacrylamide gel (described in the following section) was 5.35 times that in filter-paper discs. Therefore counts obtained on filter-paper discs were normalized by multiplication with this conversion factor (5.35).

Immunoprecipitation and sodium dodecyl sulphate polyacrylamide-gel electrophoresis of α_{2u}-globulin synthesized in vitro

^{3}H-labelled α_{2u}-globulin present within the released peptide chains was precipitated with rabbit antiserum against α_{2u}-globulin (70mg of freeze-dried antiserum in 1ml of water), after addition of carrier α_{2u}-globulin as in the procedure of Sippel *et al.* (1975), but with the modification that 200μl of the antibody solution was used for 10μg of the carrier α_{2u}-globulin. The antigen–antibody mixture containing 1% Triton X-100, 0.01M non-radioactive leucine, 0.01M-sodium phosphate (pH7.0) and 0.14M-NaCl was incubated at 37°C for 30min, followed by overnight incubation at 4°C. The antigen–antibody precipitate was washed by centrifugation through 1.0ml of 1.0M-sucrose as described by Rhoads *et al.* (1973). After removal of the sucrose the immunoprecipitate was further washed (twice) in 2.0ml of phosphate/saline by thorough mixing and re-centrifugation. The immunoprecipitate was dissolved by incubation at 100°C for 2min in 100μl of 0.625M-Tris/HCl, pH6.8, 2% sodium dodecyl sulphate, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol and 0.001% Bromophenol Blue. The dissolved immunoprecipitate was then subjected to sodium dodecyl sulphate / polyacrylamide gel electrophoresis (14%, w/v, acrylamide gel) as described by Laemmli (1970). After electrophoresis, gels were fractionated through a Gilson gel fractionator (Gilson Medical Electronics, Middleton, WI, U.S.A.), and the minced gel fractions obtained from 2mm portions of the gel were collected in scintillation vials containing 5.0ml of a counting medium of the following composition (per 100ml): 67ml of 0.5% Omnifluor in toluene, 3ml of Protosol (New England Nuclear Corp.) and 30ml of Triton X-100. The mixture was incubated for 16h at 37°C and counted for radioactivity in a Packard liquid-scintillation spectrometer.

Results

Kinetics of amino acid incorporation by the wheat-germ cell-free system under the direction of hepatic mRNA

Preliminary experiments showed an almost linear increase in the incorporation of [^{3}H]leucine into proteins with poly(A)-containing hepatic RNA at concentrations between 0 and 12μg/100μl of total reaction mixture. Maximum stimulation of [^{3}H]-leucine incorporation into proteins (15-fold over background) in the wheat-germ cell-free system was observed with 30μg of poly(A)-containing RNA/100μl of total reaction mixture. Fig. 1 shows the kinetics of incorporation of [^{3}H]leucine into total proteins by the wheat-germ cell-free system with and without addition of hepatic poly(A)-containing RNA (30μg/100μl) at 24°C. The incorporation of [^{3}H]leucine into total proteins was almost linear with time for about 60min; thereafter the amount of total protein synthesized *in vitro* declined. In the absence of added mRNA there was minimal incorporation of [^{3}H]leucine into proteins. All subsequent experiments were carried out with 60μg of poly(A)-containing RNA in 200μl reaction mixtures.

Relative amounts of circulating androgen, hepatic α_{2u}-globulin mRNA and hepatic and urinary contents of α_{2u}-globulin in male rats treated with low dosage of oestradiol-17β

Results in Fig. 2 show the effects of eight daily injections of oestradiol-17β (10μg/100g body wt.) on

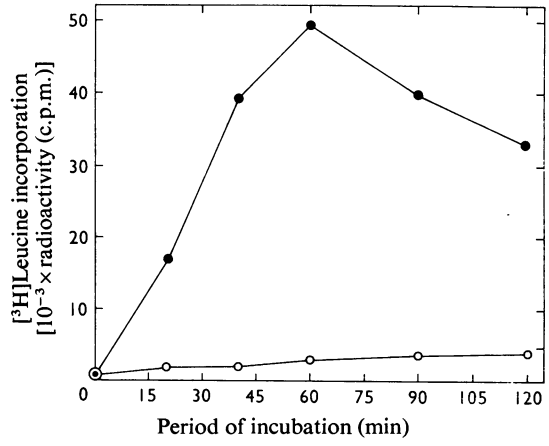

Fig. 1. *Time course of [^{3}H]leucine incorporation into proteins by the wheat-germ cell-free system*
The radioactivity represents hot (90°C)-trichloroacetic acid-insoluble precipitate in 5μl of the reaction product. ●, Incorporation after addition of 30μg of hepatic mRNA in 100μl of reaction mixture. ○, Incorporation without addition of hepatic mRNA. Each point represents an average value for duplicate experiments.

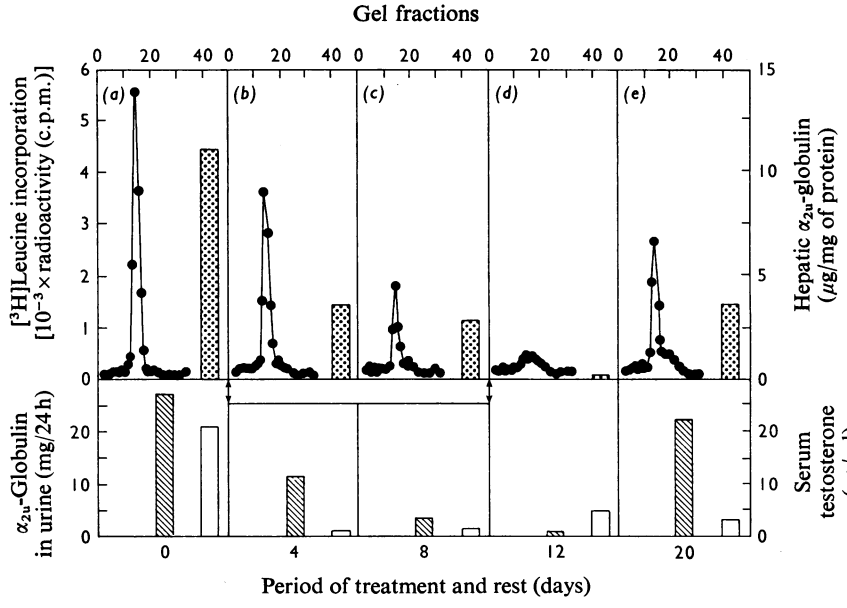

Fig. 2. *Relationship between concentrations of hepatic and urinary α_{2u}-globulin, hepatic mRNA activity for α_{2u}-globulin and serum testosterone in oestrogen-treated* (10 μg/100 g *body wt.*) *mature male rats*

Each vertical frame (*a–e*) represents average data from three experimental animals. RNA was extracted from pooled liver (3 g from each animal) and the remainder of the assays were performed separately and results are expressed as mean of the three values. (*a*), Untreated controls; (*b*) and (*c*), animals treated daily for 4 and 8 days respectively with oestradiol; (*d*) and (*e*), animals recovering for 4 and 12 days respectively after initial 8 days of daily oestradiol treatment. ●, Radioactivity pattern of the sodium dodecyl sulphate/polyacrylamide-gel electrophoretogram of α_{2u}-globulin–anti-(α_{2u} globulin) immunoprecipitate obtained from samples of released peptide chains synthesized by the wheat-germ cell-free system under the direction of hepatic mRNA containing 2×10^6 c.p.m. of incorporated [^{3}H]leucine; stippled bars, hepatic content of α_{2u}-globulin; hatched bars, 24 h urinary output of α_{2u}-globulin before death; open bars, concentrations of serum testosterone.

the concentrations of serum testosterone, hepatic mRNA for α_{2u}-globulin and the concentrations of hepatic and urinary α_{2u}-globulin. The identity of the α_{2u}-globulin synthesized *in vitro* under the direction of hepatic mRNA is authenticated by the fact that the radioactivity incorporated into the immunoprecipitated α_{2u}-globulin had the same electrophoretic mobility as pure α_{2u}-globulin (Roy *et al.*, 1976). Although the same amount (2×10^6 c.p.m.) of released peptide chain synthesized *in vitro* was used for immunoprecipitation and for subsequent electrophoresis of the immunoprecipitate in all of the experimental conditions shown in Figs. 2(*a*)–2(*e*), the fractions of radioactivity incorporated into α_{2u}-globulin varied greatly. In the untreated male rats large amounts of α_{2u}-globulin in the hepatic cytosol (11.2 μg/mg of protein) and urine (27 mg/24 h) corresponded to high radioactivity (14300 c.p.m.) in the α_{2u}-globulin fraction of the peptides synthesized *in vitro* under the direction of the hepatic mRNA from these animals. A fall of more than 50% in all of these three parameters was observed after 4 days of oestrogen treatment, and a further fall in both the mRNA activity and the urinary and hepatic concentrations of α_{2u}-globulin was observed after 8 days of oestrogen treatment. After oestrogen withdrawal, the effect of the hormone treatment still continued, and after 4 days of rest (after 8 days of oestrogen treatment) all of these three parameters reached values less than 5% of those of the untreated controls. Animals killed after 12 days of hormone withdrawal (after 8 days of oestrogen treatment) showed partial recovery from the inhibitory effect of oestrogen treatment, and the rise in the hepatic and urinary concentrations of α_{2u}-globulin coincided with the rise in the concentrations of this protein. Results in Fig. 2 also reveal that concentrations of the mRNA for α_{2u}-globulin, after oestrogen treatment and withdrawal, showed greater correlation with the daily urinary output of α_{2u}-globulin than with the hepatic concentration of this protein. Possible explanations for these differences are discussed below.

Oestrogen treatment of mature male rats also decreased the concentration of serum testosterone. However, no linear relationship between the concentrations of serum testosterone and those of α_{2u}-

Table 1. *Quantitative relationship between the concentrations of hepatic α_{2u}-globulin mRNA activity, hepatic and urinary α_{2u}-globulin and serum testosterone in mature male rats treated with oestradiol (50 μg/100 g body wt.).*

Mature male rats (350–400 g) were used for the experiment. The mRNA activity for α_{2u}-globulin is expressed as the c.p.m. of [^{3}H]leucine within the α_{2u}-globulin band after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of α_{2u}-globulin–anti-(α_{2u}-globulin) immunoprecipitate obtained from 10^6 c.p.m. of labelled peptide chains released from the polyribosomes of the wheat-germ cell-free S-30 system primed with hepatic mRNA. The urinary concentrations of α_{2u}-globulin are represented as mg of α_{2u}-globulin in 24h urine samples obtained before death. The values for the untreated control are averages for six animals; the rest of the values are averages for three experimental animals. Values less than 5% of the control are not considered significantly different from 0.

Treatment	Hepatic α_{2u}-globulin mRNA activity		Hepatic content of α_{2u}-globulin		Urinary α_{2u}-globulin content		Serum testosterone	
	Radioactivity (c.p.m.) of α_{2u}-globulin/10^6 × radioactivity (c.p.m.) of released chains	(% of control)	(μg of α_{2u}-globulin/mg of protein)	(% of control)	(mg of α_{2u}-globulin/24h)	(% of control)	(ng/ml)	(% of control)
None	7324	100	9.40	100	24.80	100	24.10	100
4 days of oestradiol	240	<5	0.34	<5	0.20	<5	3.80	15.70
8 days of oestradiol	350	<5	0.05	<5	0.50	<5	1.60	6.60
8 days of oestradiol+4 days of rest	0	0	0.29	<5	0.65	<5	0.40	<5
8 days of oestradiol+12 days of rest	150	<5	0.09	<5	0.00	0.00	0.60	<5
8 days of oestradiol+33 days of rest	2424	33	2.30	53.50	7.68	30.10	9.20	38.10

globulin and its mRNA could be observed. In all of the oestrogen-treated animals the concentrations of serum testosterone remained below 25% of those in the untreated control, yet the concentrations of α_{2u}-globulin and its corresponding mRNA varied from less than 5% to more than 50% of the control values.

Effect of treatment with high dose of oestrogen and resulting insensitivity of α_{2u}-globulin and its mRNA to androgens

Unlike use of a low oestradiol dose (10 μg/100 g body wt.), eight daily treatments with a high dose of oestradiol (50 μg/100 g body wt.) are known not only to inhibit completely α_{2u}-globulin synthesis but also to promote insensitivity to androgenic induction for about the next 4 weeks (Roy *et al.*, 1975). This suggests that higher doses of oestrogen may involve additional factors which are currently not understood. Therefore we have investigated the effect of eight daily treatments with oestradiol (50 μg/100 g body wt.) on the contents of serum testosterone, urinary α_{2u}-globulin, hepatic α_{2u}-globulin and the hepatic mRNA activity for this protein. Results are summarized in Table 1. Similar to the results obtained with the low-dose oestradiol treatment (10 μg/100 g body wt.), the results with the higher dose of the hormone also showed a striking correlation between the hepatic content of α_{2u}-globulin mRNA and the daily urinary output of α_{2u}-globulin. Changes in the serum concentrations of testosterone in the oestrogen-treated male rats showed general correlation with the changes in the α_{2u}-globulin-synthesizing activity of the hepatic tissue, as indicated by the amount of hepatic mRNA for α_{2u}-globulin and the urinary and hepatic amounts of this protein. A separate group of three male rats was also treated with eight daily injections of oestradiol (50 μg/100 g body wt.), and after 4 days of rest was again treated with testosterone (250 μg/100 g body wt.) for another 8 days. After the last testosterone treatment a 24h urine sample was collected, and the animals were then killed for the analysis of the hepatic content of α_{2u}-globulin and its corresponding mRNA. The results showed that testosterone treatment after oestradiol treatment did not induce α_{2u}-globulin, and no mRNA for α_{2u}-globulin could be detected in the liver of these animals. The above dose of testosterone is known to cause maximum induction of α_{2u}-globulin in the spayed female rat (A. K. Roy, unpublished work).

Discussion

Under the conditions of oestrogenic stimulation or withdrawal, a correlation between the amounts of α_{2u}-globulin and its corresponding mRNA has been established. However, the correlation between the daily urinary output of α_{2u}-globulin and the amount

of hepatic mRNA for α_{2u}-globulin was more striking than that between hepatic α_{2u}-globulin and the mRNA. Since the hepatic concentration of a secretory protein is generally determined by a balance between its rate of synthesis and rate of secretion, both of which can be subjected to independent variables, the daily urinary output of α_{2u}-globulin in this situation may reflect its net synthesis better. It is thus justifiable to conclude that oestrogenic inhibition of α_{2u}-globulin synthesis may be due to a corresponding decrease in the hepatic content of its mRNA. The above results are in concordance with the observations by Roy *et al.* (1975) and Kurtz *et al.* (1976). Earlier studies have also shown that oestradiol does not inhibit translation of α_{2u}-globulin mRNA in perfused rat liver (Roy *et al.*, 1975). All of these observations indicate that the effect of oestrogens on the synthesis of α_{2u}-globulin is achieved by inhibition of transcription of the α_{2u}-globulin gene. However, the possibility of post-transcriptional modulation by oestrogens cannot be overlooked.

A decrease in the concentration of serum testosterone by oestrogens, and especially maintenance of the decreased concentration of this hormone even after withdrawal of the oestrogen treatment, may provide a second means of inhibiting the synthesis of α_{2u}-globulin. Treatment of mature male rats with oestradiol at a dose of 10μg/100g body wt. does not result in a linear and correlative fall in the concentrations of serum testosterone and hepatic mRNA for α_{2u}-globulin. After 4 days of oestradiol treatment the concentration of serum testosterone fell to the lowest value, although the hepatic concentration of α_{2u}-globulin mRNA was still maintained to more than 50% of the control value. In the absence of any estimate of the half-life of α_{2u}-globulin mRNA, it could be argued that this mRNA extracted from animals after 4 days of oestrogen treatment may have been synthesized earlier when the concentration of circulating androgens was still sufficiently high. However, a similar argument cannot be justified for animals which had rested for 4 days after 8 days of oestrogen treatment. In this case the hepatic concentration of the α_{2u}-globulin mRNA was only about 5% of the control, yet the concentration of serum testosterone was as much as 23% of the control (untreated) value. Taken together with the earlier observation that simultaneous administration of both oestradiol and 5α-dihydrotestosterone blocks the androgenic induction of α_{2u}-globulin in the spayed female rat (Roy *et al.*, 1975), it appears that, although an impaired androgenic status may be a contributing factor in the inhibition of α_{2u}-globulin synthesis, it is not the primary mode of oestrogen action.

Earlier studies have shown that oestrogen treatment of mature male rats decreased and ultimately eliminated the androgen-binding protein of the liver cytosol (Milin & Roy, 1973; Roy *et al.*, 1974). The inability of the androgen treatment to induce α_{2u}-globulin mRNA within the period of temporary androgen insensitivity caused by the high-dose oestrogen treatment may therefore be due to the loss of androgen receptivity of the liver. Thus the results presented here and those published earlier (Roy *et al.*, 1974, 1975) are consistent with the concept that oestrogenic inhibition of the synthesis of α_{2u}-globulin is mediated by a decrease in the synthesis of the mRNA for this protein and that this effect may be due to an oestrogen-dependent decrease in both the sensitivity of the liver to androgens and the concentration of plasma testosterone.

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