Hiroshima Journal of Medical Sciences
Vol.35, No.4, 325~338, December, 1986
HIJM 35-48

Bioavailability, Distribution and Pharmacokinetics of Diethylstilbestrol Converted from Diethylstilbestrol Diphosphate in Patients with Prostatic Cancer

Koji NAKAMURA

Department of Urology, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

(Received August 28, 1986)

Key words: Diethylstilbestrol, Prostatic cancer, Dihydrotestosterone, Androgen receptor

ABSTRACT

A method for the radioimmunoassay of diethylstilbestrol (DES) was established to evaluate the distribution of DES in patients with prostatic carcinoma receiving intravenous infusion of 500 mg of diethylstilbestrol diphosphate (DES-DP). The plasma DES concentration was markedly elevated to 4.9 μg/ml immediately after DES-DP infusion and rapidly decreased to 0.8 μg/ml at 3 hr after the DES-DP infusion. The DES concentration in the prostatic tissue was 1.6 μg/g w.w. at 1 hr after infusion, and thereafter was predominantly similar to that in the plasma. The DES excretion in the urine was 23.7 mg at 0 to 3 hr after DES-DP infusion, and decreased gradually thereafter. In addition, the DES distribution in the subcellular fractions of benign hypertrophic prostates was studied following DES-DP infusion. The DES concentration in the nuclear fraction decreased with time. Furthermore, the protein specifically bound to DES was not detected in the cytosolic fraction. Therefore, the DES converted from DES-DP did not accumulate in the prostate. Subsequently, the bioavailabilities of DES were examined. The dihydrotestosterone (DHT) content of the prostatic tissue was significantly decreased at 24 hr after DES-DP infusion. However, the cytosolic androgen receptor content was insignificantly changed within 24 hr of infusion. Since the plasma luteinizing hormone (LH) level in the castrated patient with prostatic cancer was significantly lowered 3 hr after infusion, and further decreased with time, the main effect of DES was considered to be suppression of hypophyseal function.

Endocrine therapy is accepted to be an effective method for managing advanced prostatic carcinoma. Diethylstilbestrol diphosphate (DES-DP) has been used as a synthetic estrogenic agent was for the endocrine therapy of the patients with prostatic carcinoma. DES-DP has been postulated to be converted to diethylstilbestrol (DES) by acid phosphatase *in vivo* and this converted DES to exert its clinical effect by suppressing hypophyseal function, by decreasing free testosterone (T) in the blood via testosterone-estradiol binding globulin (TEBG) and by inhibiting the formation of 5α-dihydrotestosterone (DHT) from T in the cell of the prostate. Five hundred mg of DES-DP tended to decrease the plasma free T concentration in castrated patients with prostatic carcinoma. However, few reports on plasma and prostatic tissue DES concentration in patients with prostatic carcinoma are available. The investigation was undertaken to establish a radioimmunoassay (RIA) for DES and to evaluate the distribution of DES in the prostate. In addition, the influences of DES to the DHT content and the cytosolic androgen receptor in the prostatic tissue were also examined.

SUBJECTS AND SAMPLING

Five-hundred mg of DES-DP dissolved in saline was infused intravenously in 19 patients with prostatic carcinoma over a 2 hr period. Blood samples were obtained before and immediately after infusion, and 1, 3, 6, 12, and 24 hrs after the DES-DP infusion. In addition, prostatic tissue samples were obtained 1, 3, 6, 12 and 24 hrs after the DES-DP infusion. Meanwhile, urine samples were collected before and 0 to 3, 3 to 6, 6 to 12, 12 to 24, 24 to 48 hrs after DES-DP infusion in 11 patients with urethral catheterization.

Thirteen specimens of benign prostatic hypertrophy (BPH) were used to determine the concentrations of DES, DHT and androgen receptor contents. These samples were obtained surgically within 3 hr, and 6, 12, and 24 hrs after the DES-DP infusion. Prostatic tissues were either used freshly or frozen in liquid nitrogen and stored at −70°C until analysis.

EXPERIMENTAL PROCEDURES

1) Prostatic tissue preparation

The specimen of prostatic carcinoma was homogenized in 5 volumes of 0.25 M sucrose, the DES in the prostatic homogenate was extracted twice with 5 ml of diethylether, and then this phase was evaporated to dryness with a N_2 jet. The residue was dissolved in 1 ml of methanol. An aliquot of the methanol extract was used for DES determinations by RIA.

The specimen of BPH was homogenized in 5 volumes of buffer 1 (0.25 M sucrose, 10 mM Tris, 3 mM $CaCl_2$, pH 7.4) using glass-glass homogenizer and then was dispersed using a Dounce homogenizer (ten strokes with the loose-fitting pestle followed by five strokes with the tight fitting pestle). The homogenate was filtered through a nylon mesh. The filtrate thus obtained was centrifuged at 1,500 *g* for 10 min. The supernatant was further centrifuged at 105,000 *g* for 1 hr to obtain the cytosol fraction. On the other hand, the crude pellet was suspended in a heavy sucrose buffer (2.1 M sucrose, 10 mM Tris, 3 mM $CaCl_2$, pH 7.4) and then centrifuged at 50,000 *g* for 1 hr. To obtain the nuclear fraction, the pellet was washed twice and suspended in buffer 1. Either fraction was extracted with diethylether as for the specimen of prostatic carcinoma.

2) Measurement of DES

Materials. The tracer solution, [monoethyl-^{3}H] diethylstilbestrol (^{3}H-DES, Amersham Int. Plc., Buckinghamshire, England, S.A. 406 mCi/mg), was purified with Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) column chromatography. The labeled and unlabeled DES were stored in methanol containing 0.05% ascorbic acid. As an assay buffer, a mixture of 0.155 mol/liter NaCl and 0.1% normal rabbit serum added 0.07 mol/liter phosphate buffer (NRS-PBS) was used; and as a dextran coated charcoal reagent (DCC), a 0.5% suspension of Norit A charcoal in 0.05% dextran T-70 in saline was used. The radioactivity was counted using a liquid scintillation spectrometer (Packard Instrument Inc., Chicago).

Methods.

Preparation of anti-DES antibody. DES-carboxymethyl ether-bovine serum albumin (DES-CME-BSA) was prepared according to the method of Erlanger[16]. An emulsion of DES-CME-BSA (1 mg/ml) in saline and Freund's complete adjuvant (1:1, V/V) was used to raise the antisera in two rabbits. With the method of Ouchtelony, immunodiffusion was carried out.

Hydrolysis of conjugated DES in urine. Fifty μl of urine, 1,000 units/100μl of β-glucuronidase (Helix pomatia, Sigma, St. Louis) and 50 μl of acetate buffer (pH 5.0) were incubated at 37°C for 24 hr to hydrolize the conjugated DES.

RIA of DES. Five hundred μl of NRS-PBS, 200 μl of anti-DES antibody, 200 μl of ^{3}H-DES and 100 μl of standard DES (50 to 1600 pg of DES) or of the prepared samples were incubated at 4°C for 3 hr, and then 200 μl of DCC was added to the mixture above-mentioned. The reaction was allowed to proceed at 4°C for another 15 min. The radioactivities of the supernatants were counted. The dose-response curve obtained was plotted as a log-logit transformation.

3) Measurement of prostatic DHT content

Prostatic DHT content was measured using a Testosterone/Dihydrotestosterone RIA kit (Amersham Int. plc., Buckinghamshire, England). Briefly, the tissue of BPH was homogenized in 1 ml of 50 mM Tris buffer. The ether extract was evaporated and suspended in distilled water. The oxidation step was carried out for the measurement of DHT level. The solution was extracted again. The dried extracts

were used for DHT radioimmunoassay.

4) Characterization and quantitation of cytosolic androgen receptors in prostatic tissue.

Preparation of prostatic cytosol. The tissue specimen was homogenized with 5 volumes of TED buffer (10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol). After centrifugation at 105,000 *g* for 1 hr, the supernatant containing the cytosol was collected for further studies.

Binding assay. The incubation was carried out with the [6, 7-^{3}H]-labeled synthetic androgen methyltrienolone (17β-hydroxy-17α-methyl-4, 9, 11-estratrien-3-one, R-1881, S.A. 55.5 Ci/m mole) as ligand in the presence or absence of a 100 fold excess of unlabeled ligand. Moreover, 100 nM triamcinolone acetonide (TA) was added to the incubation media to eliminate the binding of R-1881 to progesterone receptors.

Quantitation of androgen receptor. The incubation was generally done using 7 different concentrations (approximately 0.1 nM to 5 nM) of the ligand to allow construction of Scatchard plots[13,50]. The incubation was performed over night (16 hr) at 0°C. After incubation, the samples were treated with DCC suspended in the buffer described above. Aliquots were taken for counting of radioactivity. The maximum number of binding sites (Bmax) and apparent dissociation constant (Kd) were calculated from Scatchard plots.

Characterization and isolation of androgen-receptor complex in prostatic cytosol. The system for gel filtration high performance liquid chromatograph (HPLC) analysis was a HLC-803D with a UV-8 model II as a spectrophotometer and a TSK gel G3000SW column (Toyo Soda manufacturing Co., Japan). The cytosol fractions of prostatic tissue were incubated in the presence of 10 nM of ^{3}H-R-1881 and 1 μM TA without any addition of radioinert R-1881 and in the presence of 1 μM R-1881 for 16 hr at 0°C. After free steroids were removed by treating the cytosol with 1% DCC. 0.3 ml of the aliquots filtrated through a milipore filter (0.45 μm, Gelman science, Japan) were applied for HPLC analysis. The column was maintained at 0°C, 0.07 mol/liter phosphate buffer (pH 7.4) was collected to count the radioactivity of each fraction (0.35 ml). Furthermore, as marker proteins, a MW-Marker (HPLC) (M.W. 12,400—290,000, Oriental Co., Japan) was used to determine the sedimentation coefficients(S) of the androgen-receptor complex.

5) Characterization and isolation of specific binding protein to DES in prostatic cytosol

The incubation was carried out with 100 nM ^{3}H-DES in the presence or absence of a 100-fold excess of unlabeled DES for 16 hr at 4°C. After free steroids were eliminated, an aliquot was applied to examine the specific binding protein to DES by means of the above-mentioned HPLC likewise androgen receptor analysis. In addition, an aliquot was applied to sucrose density gradient analysis (SDG). 0.2 ml of the aliquot of a clear supernatant was layered onto 5 to 20% linear sucrose gradients containing 10 mM Tris, 1.5 mM EDTA and 10% glycerol at pH 7.4. Gradients were formed in 3.0 ml cellulose nitrate tubes using a gradient former (Densi-Flow IIc, Buchller Instruments, Fort Lee, N.J.). SDG was then centrifuged at 230,000*g* for 12 hr at 0°C. Fractions (0.0 ml) were collected from the top of each tube for radioactivity estimation. Bovine serum albumin (4.6S) and γ-globulin (7.1S) were used as marker proteins to estimate sedimentation coefficients.

6) Measurements of serum T and LH concentrations

Serum T levels were determined using a Testosterone Direct RIA-kit (Commissariat A L'Energie Atomique, Italy) with 7.2% cross-reactivity to DHT. Since T was not separated from DHT before the assay, the evaluated T levels may contain small amounts of DHT.

Serum LH levels were also determined using a LH I-125 kit (Commissariat A L'Energie Atomique).

7) Other experiments

Protein concentrations in the subcellular fractions were measured according to the method of Lowry[39]. Furthermore, DNA concentrations were measured by the method of Labarca[34].

STATISTICAL ANALYSIS

All numerical values were expressed as mean ± standard deviation (SD). Student's t-test was used to compare differences in values between the means of the experimental groups.

RESULTS

1) RIA of DES

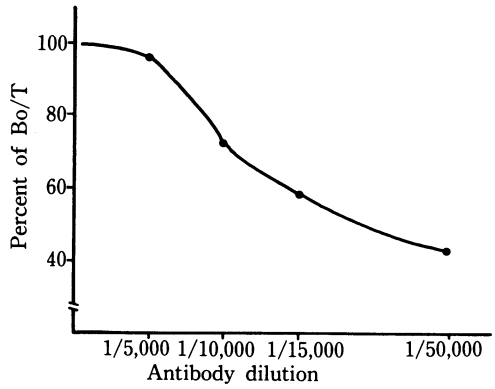

Fig. 1. Anti-DES carboxymethyl ether-BSA dilution curve

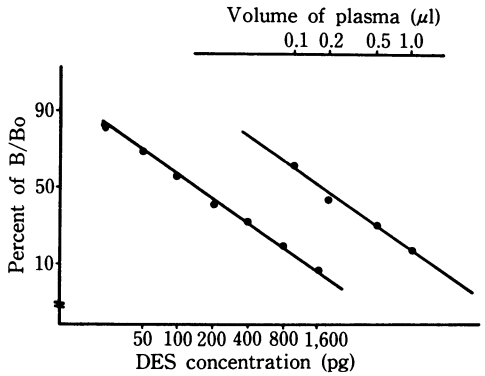

Fig. 2-a. The standard curve for DES and the dilution curve of the plasma

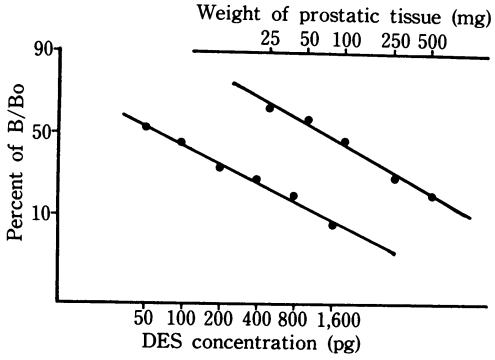

Fig. 2-b. The standard curve and the dilution curve of the extract of prostate

With a percent binding of ^{3}H-DES radioligand to antiserum, the titration curve indicated that the antiserum was suitable for measuring DES by RIA at a level of dilution of 1:15,000 (Fig. 1). Figure 2 shows the standard and dilution curves obtained. The straight line was obtained between 50 and 1600 pg of DES, and the dilution curves of the plasma, the extract of the prostate and the hydrolized urine were in parallel with the standard curve (Fig. 2-a,b,c). By the addition of an antibody diluted

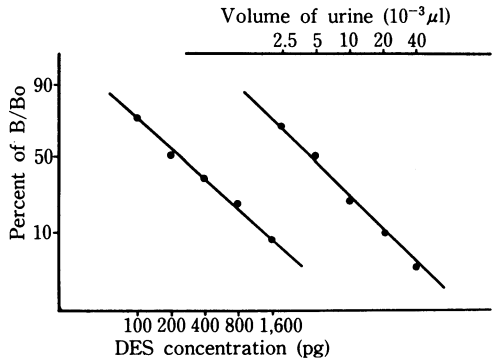

Fig. 2-c. The standard curve and the dilution curve of the urine hydrolized with β-glucuronidase

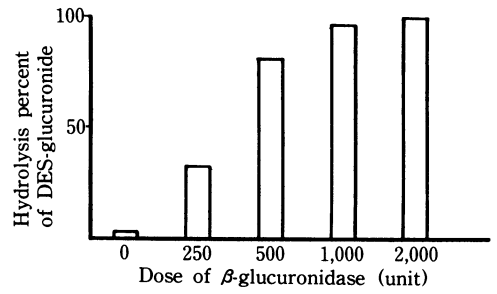

Fig. 3. Hydrolysis percent of the conjugated DES in the urine on the condition of incubation at 37°C for 24 hr

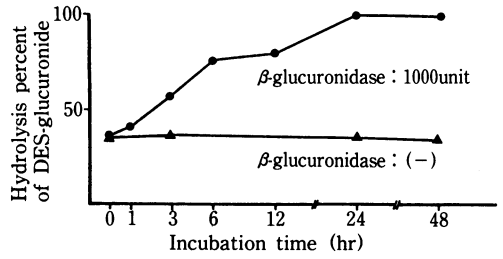

Fig. 4. Changes of hydrolysis percent of the conjugated DES in the urine on the condition of incubation with 1,000 units of β-glucuronidase at 37°C

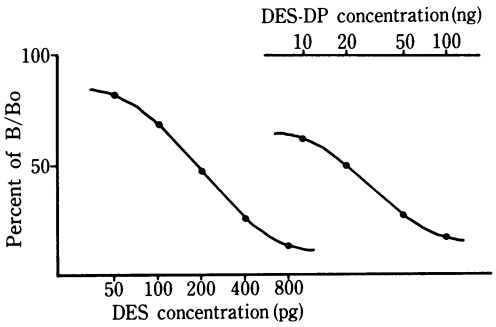

Fig. 5. The standard curve for DES and inhibition curve of DES-DP over the range 10 to 100 ng

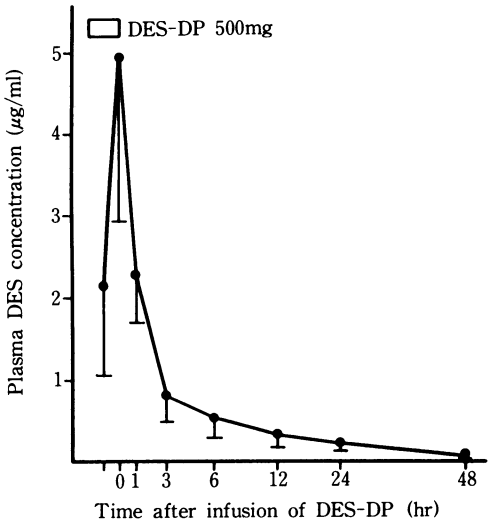

Fig. 6. Plasma DES concentrations in 19 patients with prostatic carcinoma following intravenous drip infusion of DES-DP

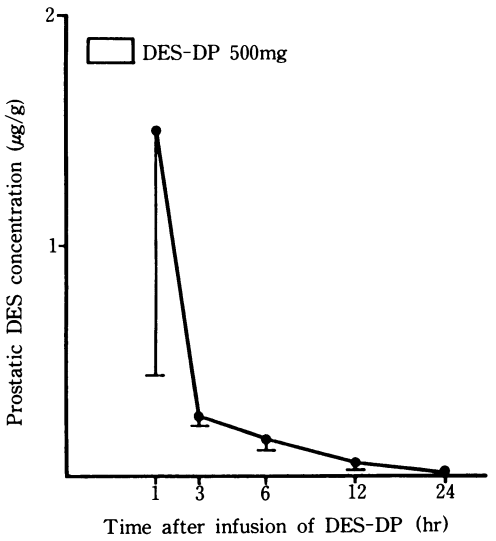

Fig. 7. Prostatic DES concentrations in patients with prostatic carcinoma following intravenous drip infusion of DES-DP

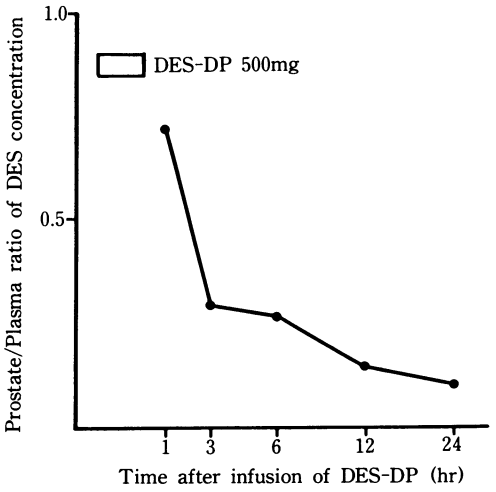

Fig. 8. Changes of prostate/plasma ratio of DES concentration following DES-DP infusion

to 1:15,000, an amount of DES as small as 40 pg per tube could be detected in this assay system, and 0.1 μl of plasma obtained from a patient at 3 hr after infusion was sufficient to determine the DES concentration.

The percent of hydrolysis on the conjugated DES in the urine was 96% at 24 hr (Fig. 3). The dose of β-glucuronidase between 1,000 units and 2,000 units did not influence the percent of hydrolysis.

DES concentrations determined by RIA in the urine sample were gradually increased until 24 hr after the start of hydrolysis (Fig. 4).

The intra-assay coefficient variation by nine-plicate assays was 6.2% and the inter-assay coefficient variation by 5 different assays was 14.9%.

The cross-reaction of the anti-DES antibody with the steroids, testosterone, estradiol and ethynylestradiol, was less than 0.01% the reaction with DES, however, that with DES-DP was 0.7% (Fig. 5).

2) DES concentrations in plasma and prostatic tissue following DES-DP infusion.

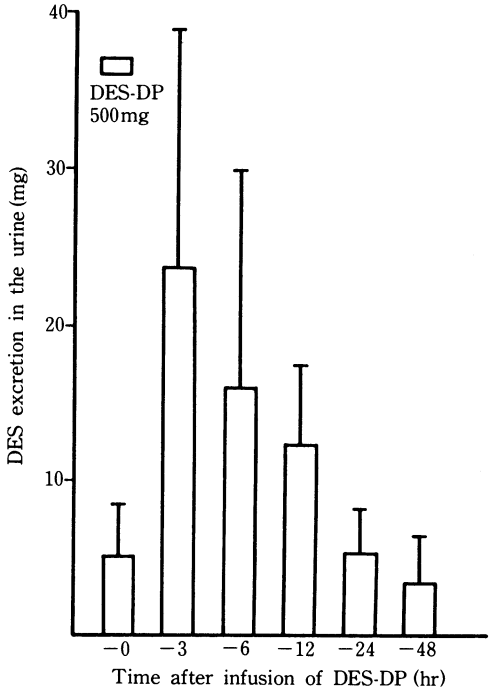

Fig. 9. DES excretions in urine of 11 patients with prostatic carcinoma following intravenous drip infusion of DES-DP

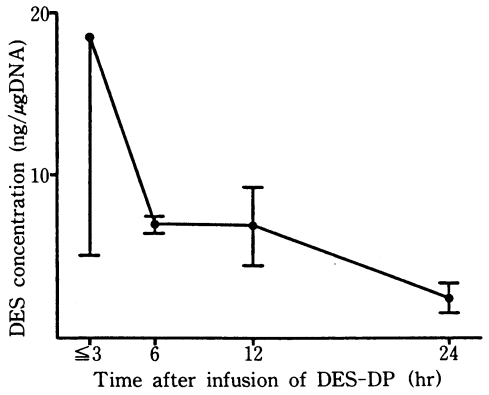

Fig. 10-a. DES concentrations in the prostatic cytosol fractions following intravenous drip infusion of DES-DP

Plasma concentrations of DES were elevated markedly to 4.9 μg/ml immediately after the termination of infusion and decreased rapidly to 0.8 μg/ml at 3 hr after the infusion. Then, the plasma DES concentrations were decreased gradually to 0.2 μg/ml at 24 hr after infusion (Fig. 6).

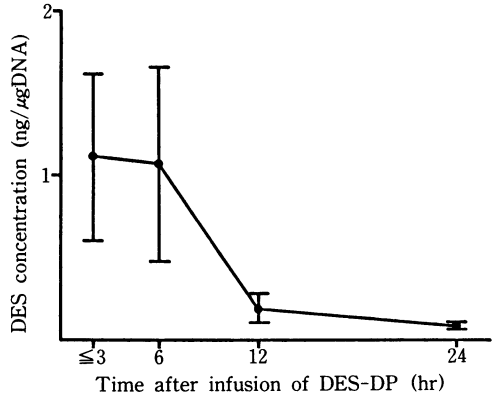

Fig. 10-b. DES concentrations in the prostatic nuclear fractions following intravenous drip infusion of DES-DP

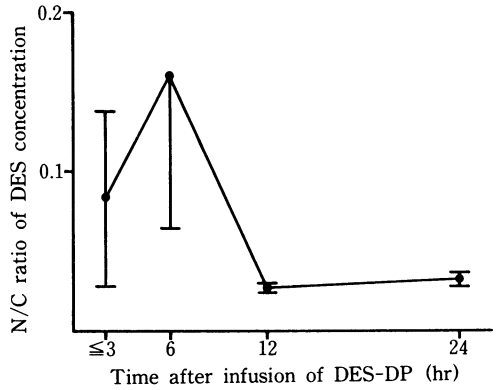

Fig. 11. Changes of prostatic nuclear/cytosol ratio of DES concentration following DES-DP infusion

The DES concentration in the prostatic tissue was 1.6 μg/g wet weight (w.w.) at 1 hr, and rapidly decreased to 0.25 μg/g w.w. at 3 hr after the infusion. The decrease in DES concentration in the prostatic tissue was predominantly similar to that in the plasma (Fig. 7).

The tissue/plasma ratio of DES concentration was decreased rapidly within 3 hr, and then gradually decreased until 24 hr after DES-DP infusion (Fig. 8).

3) Excretion of DES in urine following DES-DP infusion (Fig. 9).

DES excretion in the urine was 23.7 mg at 0 to 3 hr after DES-DP infusion. Later, DES excretion was decreased gradually to 5.3 mg

from 12 to 24 hr after infusion. DES was still detected in the urine 24 to 48 hr after infusion.

4) The DES concentration in subcellular fractions of prostatic tissue following DES-DP.

The DES concentration in the cytosolic fraction was 18.5 ± 13.4 ng/μg DNA within 3 hr of DES-DP infusion, and then declined rapidly to 6.9 ± 0.5 ng/μg DNA at 6 hr after infusion. The change in the cytosolic DES concentration was gradually decreased with time thereafter. However, the DES in the cytosol was still detected 24 hr after infusion (Fig. 10-a).

Meanwhile, the DES concentration in the nuclear fraction was 1.12 ± 0.51 ng/μg DNA within 3 hr of DES-DP infusion. At 6 hr after infusion, the nuclear DES concentration was the same as that within 3 hr of infusion. The nuclear DES concentration was decreased rapidly thereafter (Fig. 10-b).

Prostatic nuclear/cytosol ratio of DES concentration was momentarily increased at 6 hr after infusion, but, this ratio was gradually decreased thereafter with time (Fig. 11).

5) Effects of DES to DHT contents of prostatic tissue (Table 1)

In the 5 specimens of BPH without infusion of DES-DP, the DHT content of the prostatic tissue was 5.92 ± 1.27 ng/g w.w..

In the 13 specimens of BPH with infusion of DES-DP, the DHT content was 6.10 ± 1.30 within 3 hr, 5.42 ± 0.66 at 6 hr, and 4.81 ± 0.93 ng/g w.w. at 12 hr after DES-DP infusion.

Table 1. Changes of prostatic DHT contents following DES-DP infusion

Time after infusion (hr)	No. of pts.	DHT (ng/g w.w.)
Without DES-DP	5	5.92 ± 1.27[a)]
≦3	4	6.10 ± 1.30
6	3	4.81 ± 0.93
12	3	5.42 ± 0.66
24	3	3.92 ± 0.17[b)]

a vs. b : significantly different by $p<0.05$

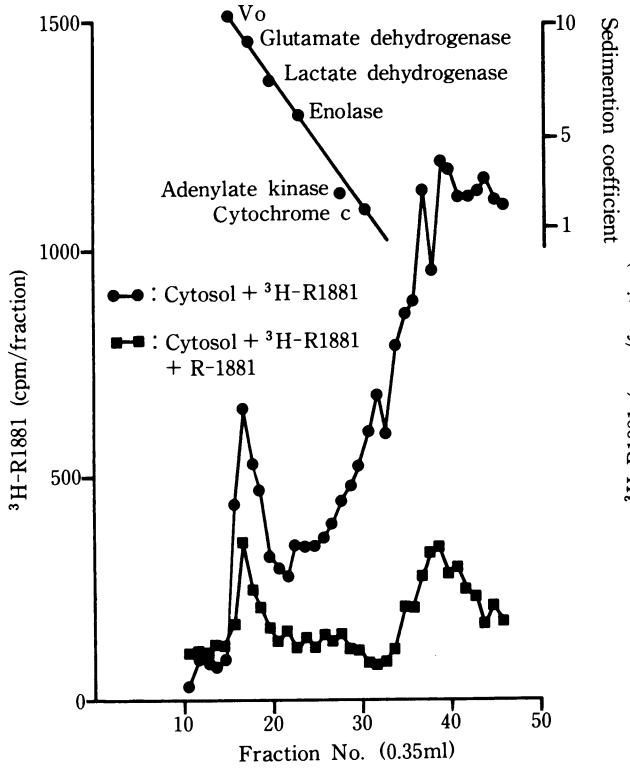

Fig. 12-a. The HPLC pattern of R-1881 binding in prostatic cytosol from a patient with BPH

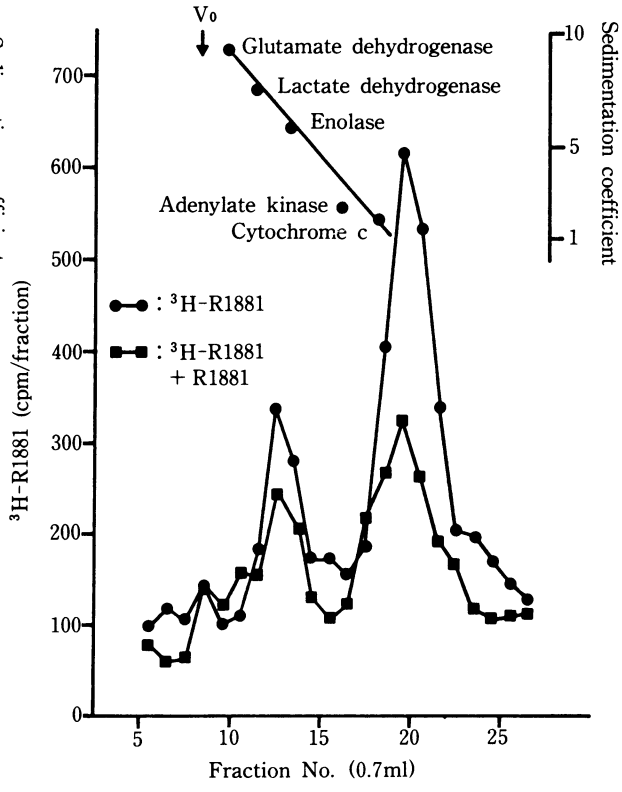

Fig. 12-b. The HPLC pattern of R-1881 binding in prostatic cytosol from a patient with BPH following DES-DP infusion

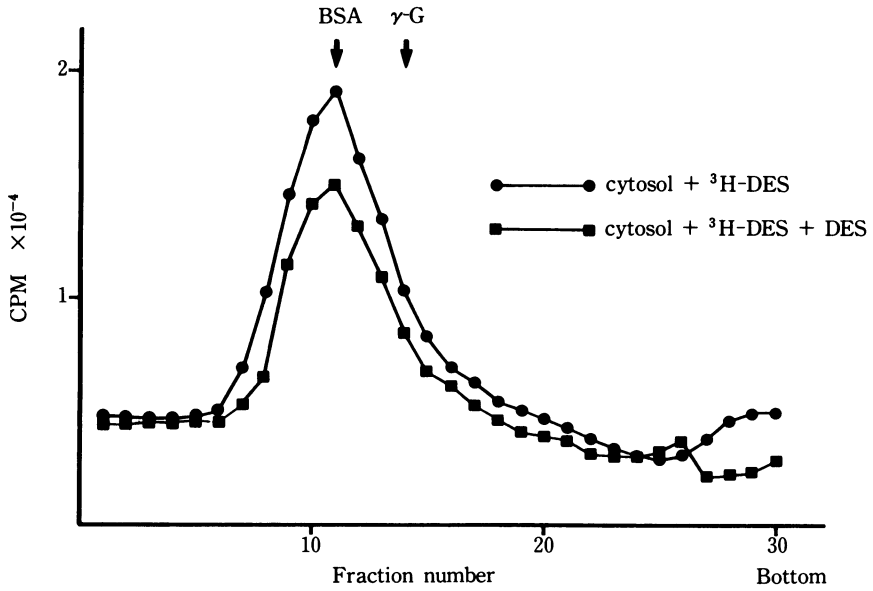

Fig. 13. The sucrose density gradient pattern of DES binding in prostatic cytosol from a patient with BPH

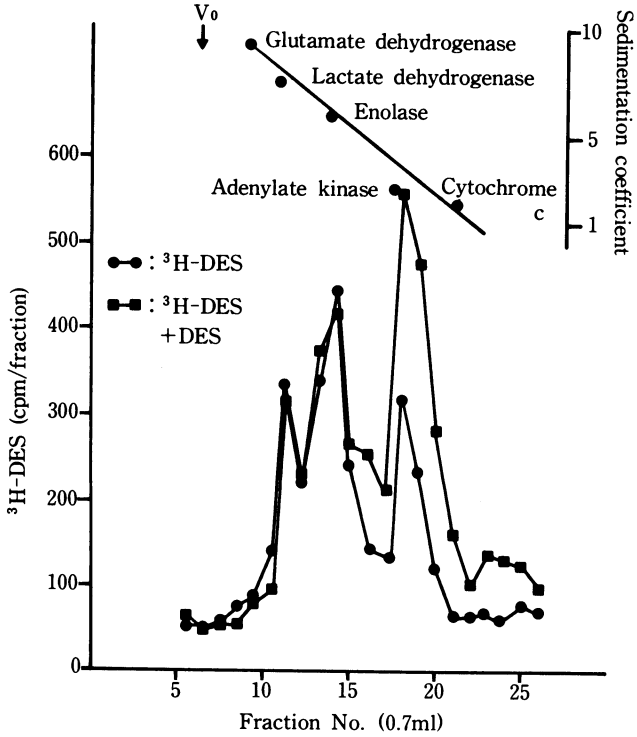

Fig. 14. The HPLC pattern of DES binding in prostatic cytosol from a patient with BPH

Table 2. Changes of cytosolic androgen receptor contents (Bmax) in the prostates following DES-DP infusion

Time after infusion (hr)	No. of pts.	Bmax (fmol/mg protein)
Without DES-DP	4	343 ± 97
≦3	3	294 ± 50
6	3	340 ± 86
12	3	363 ± 99
24	3	377 ± 79

DHT contents were insignificantly changed within 12 hrs, but, the contents were significantly decreased to 3.92 ± 0.17 ng/g w.w. at 24 hr after infusion.

6) Effects of DES to cytosolic androgen receptor contents in prostatic tissue (Table 2)

The mean Bmax as the cytosol receptor contents was 343 ± 97 fmol/mg protein in 4 specimens of BPH without infusion of DES-DP. In 12 specimens of BPH with prior infusion of DES-DP, the mean Bmax was 294 ± 50 fmol/mg protein within 3 hr of infusion, but, the Bmax was insignificantly changed with time compared to that without infusion of DES-DP.

7) Influence of DES to androgen receptor complex in prostatic cytosol

The HPLC pattern of R-1881 binding in the cytosol obtained from a patient with BPH incubated with 10 nM of ^{3}H-R-1881 demonstrated a distinct R-1881 binding protein. The addition of 100 fold excess of unlabeled R-1181 abolished the radioactive peak indicating the presence of specific R-1881 binding protein (Fig. 12-a).

In contrast, the HPLC pattern on the cytosol from a BPH removed at 3 hr after DES-DP infusion was similar to that without any DES-DP infusion (Fig. 12-b).

8) Characterization and isolation of specific binding protein to DES in prostatic cytosol.

The SDG pattern of the DES binding in the cytosol obtained from a patients with BPH indicated the presence of a nonspecific binding protein with a sedimentation coefficient of approximately 4.5S which corresponded with the peak of BSA. However, the simultaneous addition to the incubation medium of 10 μM of unlabelled DES as a competitor reduced the peak of radioactivity (Fig. 13).

Figure 14 illustrates the HPLC pattern of cytosol obtained from the same patient. Two radioactive peaks of ^{3}H-DES corresponded with approximately 5.8S and 9.1S. However, the addition to the incubation medium of 10 μM of unlabeled DES had no effect on the absence of a detectable specific DES binding protein under this experimental condition.

9) Effect of DES to plasma LH and T levels in the patients with prostatic carcinoma after castration

Plasma LH and T levels were measured in 7 of 19 patients whose blood was sampled to determine their plasma DES concentrations.

Before infusion of DES-DP, the average plasma LH level was elevated to 206.4 ± 28.4 mIU/ml. The LH level was significantly lowered to 150.2 ± 20.6 mIU/ml at 3 hr after infusion, and further decreased with time. At 48 hr after infusion, the LH level was suppressed to 90.0 ± 16.4 mIU/ml (Table 3).

The average plasma T level was decreased to 0.49 ± 0.12 ng/ml before DES-DP infusion. Plasma T levels were insignificantly changed with time after DES-DP infusion compared with the level before infusion (Table 4).

Table 3. Changes of plasma LH levels in 7 patients with prostatic carcinoma following DES-DP

Time after infusion (hr)	Plasma LH concentration (mIU/ml)
Before	206 ± 28[a)]
Immediately after	169 ± 37
3	150 ± 21[b)]
6	123 ± 27
12	143 ± 30
24	117 ± 15
48	90 ± 16

a vs. b : significantly different by $p<0.05$

Table 4. Changes of plasma testosterone levels in 7 patients with prostatic carcinoma following DES-DP infusion

Timer after infusion (hr)	Plasma testosterone concentration (ng/ml)
Before	0.49 ± 0.12
Immediately after	0.57 ± 0.27
3	0.44 ± 0.13
6	0.51 ± 0.25
12	0.50 ± 0.19
24	0.41 ± 0.17
48	0.43 ± 0.22

DISCUSSION

For measuring the DES concentration, both thin layer chromatographic-fluorimetric[31,51,61] and gas chromatographic methods[37,58] have been employed. RIA for DES is also sufficient to measure clinical materials because of its simplicity and sensitivity[1,26,31,35]. The anti-DES antibody used in this study did not cross-react with some endogeneous sex steroid hormones, i.e., testosterone and estradiol, and the sensitivity of this assay system was sufficient to detect 40 pg of DES at a final antibody dilution of 1 : 15,000. In addition, the cross-reaction of this antibody with DES-DP was only 0.7% of the reaction with DES. The antibody was regarded as having high specificity and sensitivity to DES. The validity of this assay for the measurement of DES in plasma without ether extraction was proved. Moreover, the ether extract of DES in plasma and prostatic tissue could be measured by RIA without prior hydrolysis, since the dilution curve of the extract was parallel with the standard curve of DES. Therefore, we believe that DES-DP was metabolized to DES as an active form in the plasma and the prostatic tissue at the early phase following DES-DP.

DES-DP is metabolized to DES through DES-monophosphate and finally DES is metabolized to DES-monoglucuronide and - sulphate[10,47]. Using radiolabeled DES, the liver was found to be the major site of accumulation of DES and its metabolites in mice since the total radioactivity in this organ accounted for 50% of the injected dose within 2.5 min of treatment[52]. Other investigators also obtained similar results in rat and humans[19,43]. DES-glucuronide in the liver excreted into the intestine through the bile was hydrolized to unconjugated DES by intestinal bacterial β-glucuronidase, and then some of the DES was recirculated in blood through the portal vein, and also transfered into the enterohepatic circulation[18,19,27]. The plasma DES concentration at 24 hr after the infusion was judged to be 4% of that at the termination of DES-DP infusion.

The maximum DES concentration in the plasma was 3.6 μg/ml after a 1 hr infusion of 1,000 mg of DES-DP[2] and about 2.5 μg/ml after a 3.5 hr infusion of 1,500 mg of DES-DP[47]. High concentrations were supposed to induce objective responses in patients refractory to conventional oral DES therapy[2]. The plasma concentration of DES produced after a 1,000 mg infusion of DES-DP was 1,500 times the DES concentration produced by conventional oral DES doses[2]. After a 2 hr infusion of 500 mg of DES-DP in men, the maximum DES concentration was 4.9 μg/ml immediately after DES-DP infusion. The plasma DES concentration was clearly higher than that reported previously[2,47]. This may be due to the different methods of DES measurement and the different patients examined. The elimination of DES from the plasma was quite rapid and the plasma DES concentration at 3 hr post-dosing was 16% of that at 0 hr post-dosing. This observation supported the report that the DES produced from DES-DP was rapidly declined from the plasma and t1/2 for DES following administration of DES was 80 ± 10 min[2]. Sheehan et al demonstrated a lack of high affinity binding of DES to human TEBG[55]. DES bound more strongly than E_2 to normal plasma, conversely, much more weakly to the high affinity binding protein found in humans. The competition studies with TEBG suggested that DES was a weak binder[46,49]. From the above-mentioned facts, the DES converted from DES-DP in serum did not bind to TEBG, thus the plasma DES quickly decays after it is metabolized in liver.

Fifty eight to 85% of the urinary metabolites of synthetic estrogen as ethynyl-estradiol (EE2) was reported to be hydrolyzable with β-glucuronidase, while the sulphate conjugate comprised 8.4 to 11.8%[30]. Meanwhile, approximately 20% of the administered radioactivity was excreted in the urine after a single small oral dose of ^{3}H-DES in steers[44]. In another study, about 60% of the total radioactivity in the glucuronide fraction of 24 hr urine was DES glucuronide after administered ^{3}H-DES in humans[42]. DES excretions in the urine of cattles were detectable up to 22 days after intramuscular treatment with DES[51]. As determined by RIA, DES excreted into the urine was detectable with prior hydrolysis by β-glucuronidase. Most of the glucuronized DES was hydrolyzable with β-glucuronidase likewise other synthetic estrogens. DES excretion into the urine within 3 hr following DES-DP infusion was about 48% of the total DES excretion until 24 hr. DES excretion into the urine is rapid

after administration of DES-DP.

On the results of rats, prostatic tissue concentrations of DES converted from DES-DP did not appear to liberate or to accumulate DES selectively in the prostate[2]. Neither study was carried out for the determination of DES concentrations in human prostatic tissue following DES-DP infusion. RIA for DES allows the measurement of a trace amount of DES[6]. Therefore, we could measure the tissue/plasma ratio of DES concentration after the termination of infusion. The data we obtained on humans coincided with the results obtained with the rat[2].

Though DES in known to liberate DES in the phosphatase-rich environment of the prostatic tissue, DES in not considered to accumulate selectively into the prostate of man.

The change in the DES concentration of the total prostatic tissue was almost similar to not only to that of the cytosolic but also the nuclear DES concentration. In addition, except for increasing temporarily at 6 hr after infusion the nuclear/cytosol ratio of the DES concentration decreased with time. Also, the sedimentation pattern of DES binding in the cytosol indicated the absence of a specific binding protein with approximately 4.5S with corresponded to the peak of BSA on the SDG and HPLC analysis. These results indicate that no accumulation of DES occurs in the prostatic nuclear fraction and that DES was merely perfused into the prostatic nuclei.

On the other hand, only 10% of the prostatic tissue from men under the age of 65 were estrogen receptor positive, whereas nearly 80% of those over the age of 75 were estrogen receptor positive[59]. Furthermore, some authors have suggested the existence of an estrogen receptor in human prostatic tissue[8,24], but others have failed to demonstrate its presence[25,33,53]. Ekman et al indicated that the lack of cytosolic estrogen receptors in hyperplastic prostates might be caused by intranuclear accumulation of an estrogen receptor complex[15]. However, the studies of cytosolic estrogen receptor were performed by using 17β-^{3}H-estradiol and unlabeled DES as a competitor[4,8,24]. Whether a specific binding protein to DES exists in the human prostatic tissue still remains to be studied.

The specific receptor for DHT is known to be present in the prostatic cells and DHT as an active form of androgen could influence the growth of the prostate. The accumulation of DHT in BPH depends upon the increase of 5 α-reductase activity[11,45]. On the other hand, the prostatic DHT content has an influence on the 5α-reductase, 17β-hydroxysteroid dehydrogenase and 3α-hydroxysteroid oxidoreductase[6,41]. Estradiol inhibits a process of 5α-reduction and stimulates the process of 17β-dehydrogenation in the prostate[6,28]. These effects have been explained to be direct actions of DES to the prostate. Conversely, Lee et al demonstrated that T had only a negligible effect on DHT in the prostatic tissue[36]. Intravenously administered DES-DP in humans dose not appear to act via the inhibition of 5α-reductase into the prostatic cells for producing the clinical improvement[60]. However, we found that the prostatic DHT content was decreased within 24 hr of DES-DP infusion. This activity has been reported to be unreliable as a biochemical indicator of prostatic carcinoma since the response of antiandrogenic hormone therapy to prostatic carcinoma is inconsistent with 5α-reductase activity[20]. These results indicate that the DHT content reduced by DES-DP infusion is not associated with the inhibition of DES to 5α-reductase activity. Furthermore, the direct action of DES in human prostate may not be mediated through the inhibition of 5 α-reductase activity *in vivo*.

DES treatment has been shown to increase the prostatic androgen receptor concentration in patients with BPH compared to untreated patients[32]. In addition, a single dose (35 μg/rat) of estradiol to intact rats could increase the prostatic cytosolic ^{3}H-R-1881 binding at 24 hr after injection[9]. Since the ability of estrogens to increase the androgen receptor concentration was closely related to their estrogenic potency, the increase could be mediated by the estrogen receptor presented in the prostate[9]. The androgen receptor has been considered to increase due to a lowering of the plasma T concentration caused by the effect of DES on the testicular synthesis of androgen[14,56]. In addition, it is well documented that DES substantially reduces plasma T levels by inhibiting gonadotrophin production as well as stimulating TEBG in man[29]. DES does not have any inhibitory effect on the

cytosolic binding of DHT to the receptor[21,57]. Furthermore, DES has been reported to have only a slight effect on the nuclear retention of DHT even at a 500 times higher concentration[17,38]. In this investigation, the cytosolic androgen receptor contents with DES-DP infusion insignificantly differed from those without infusion. Furthermore, the receptor contents were not correlated with the changes in cytosolic DES concentration until 24 hr after DES-DP infusion. In addition, on the elution pattern of HPLC, the binding site of ^{3}H-R-1881 to the androgen receptor and the radioactivity of specific binding in the prostate with DES-DP infusion were not clearly changed compared to that without infusion. Therefore, we suggest that a single infusion of DES-DP does not influence directly the cytosolic androgen receptor.

The change of T and some of its metabolites following the treatment of patients with DES has been examined by various worker[12,22,23,54]. Almost all of them have reported that the circulating T level declined significantly. The use of 3 mg of DES per day reduced the level of T to approximately that of the castrated male[22]. The mean percentage for suppression of serum T in the patients receiving 3 mg of DES per day was 96.8% and the corresponding value in orchiectomized patients was 98.2%[22]. Basically, similar results have been reported by other investigators[23,54]. Serum T levels were decreased to 180-870 pg/ml in patients with prostatic carcinoma after castration[5,7,40]. In addition, it was reported that serum T were further reduced to about 100 pg/ml following administration of DES in castrated patients[48]. However, in this study, serum T in the castrated patients was not altered following DES-DP infusion. From the above findings, serum T levels were completely inhibited 1 to 2 weeks after castration, and the T levels did not further declined following DES-DP infusion. On the other hand, serum LH levels elevated after castration were significantly decreased immediately after DES-DP infusion, and remained so until 48 hr after infusion. The marked decrease in LH levels seen with DES administration is consistent with the results obtained by a number of investigators[3,5,12,23]. Thus, the hypophyseal function is inhibited immediately after single administration of DES-DP.

In conclusion, DES converted from DES-DP did not accumulate selectively into the prostate. It was speculated that the main effect of DES was suppression of hypophyseal function.

ACKNOWLEDGEMENTS

I thank Professor H. Nihira for instruction and critical review of the manuscript. I also thank Associate Professor T. Usui and Professor T. Ishibe of Department of Urology, Shimane Medical University and Professor Y. Miyachi of Department of Internal Medicine, Toho University School of Medicine for invaluable advice. I am indebted to Associate Professor A. Kambegawa of Department of Gynecology, Teikyo Medical University for DES-CME-BSA and A. Yasukawa of Department of Urology, Kagawa Medical School for performing HPLC.

REFERENCES

1. **Abraham, G.E., Reifman, E.M., Buster, J.E., Stepheno, J.D. and Marshall, J.R.** 1972. Production of specific antibodies against diethylstilbestrol. Anal. Lett. **5**: 479—486.
2. **Abramson, F.P. and Miller, H.P.** 1982. Bioavailability, distribution and pharmacokinetics of diethylstilbestrol produced from stilphostrol. J. Urol. **128**: 1336—1339.
3. **Alder, A., Burger, H.G., Davis, J., Dulmanis, A., Hudso, B., Sarafaty, G. and Straffon, M.G.** 1968. Carcinoma of prostate: Response of plasma luteinizing hormone and testosterone to oestrogen therapy. Br. Med. J. **1**: 28—32.
4. **Auf, G. and Ghanadian, R.** 1982. The characterization and measurement of cytoplasmic and nuclear oestradiol-17β receptor proteins in benigh hypertrophic prostate. J. Endocrinol. **93**: 305—317.
5. **Baker, H.M.G., Burger, H.G., De Krester, D.M. Hudson, B., Rennie, G.C. and Staffon, W.G.** 1975. The assessment of results following endocrine therapy for prostaic cancer. J. Urol. **113**: 824—828.
6. **Bard, D.R. and lasnitzki, I.** 1977. The influence of estradiol on the metabolism of androgens by-human prostatic tissue. J. Endocr. **74**: 1—9.
7. **Bartsch, W., Horst, H.J., Becker, H. and Nebuse, G.** 1977. Sex hormone binding globulin binding capacity, testosterone, 5α-dihydrotestosterone, oestradiol and prolactin in plasma of patients with prostatic carcinoma under various types of hormonal treatment. Acta Endocrinol. **85**: 650—664.
8. **Bashirelani, N., O'Toole, J.H. and Young, J.D.** 1976. A specific 17β-estradiol receptor in human benign hypertrophic prostate. Biochem. Med. **15**: 254—260.

9. **Bouton, M.M., Pornin, C. and Grandadam, J.A.** 1981. Estrogen regulation of rat prostate androgen receptor. J. Steroid Biochem. **15**: 403—408.
10. **Bradford, P.A., Olavesen, A.H., Curtis, C.G. and Powell, G.H.** 1977. Metabolic fates of diethylstilboestrol sulphates in the rat. Biochem. J. **164**: 423—430.
11. **Bruchovsky, N. and Lieskovsky, G.** 1979. Increased ratio of 5 α-reductase: 3α (β) hydroxysteroid dehydrogenase activities in the hyperplastic human prostate. J. Endocrinol. **80**: 289—301.
12. **Celler, J., Albert, J., Yen, S.S.C., Geller, S. and Loza, D.** 1981. Medical castration of males with megestrol acetate and small doses of diethylstilbestrol. J. Clin. Endocrinol. Metab. **52**: 576—580.
13. **Chammness, G.C. and McGuire, W.L.** 1975. Scatchard plots: Common errors in correction and interpretation. Steroids **26**: 538—542.
14. **Danutra, V., Harper, M.E. and Griffiths, K.** 1973. The effect of stilboestrol analogues on the metabolism of steroids by the testis and prostate of the rat in vitro. J. Endocr. **66**: 539—544.
15. **Ekman, P., Snochowski, M., Dahlberg, E., Bression, D., Hogberg, B. and Gustafsson, J-Å.** 1979. Steroid receptor content in cytosol from normal and hyperplastic human prostates. J. Clin. Endocrinol. Metab. **49**: 205—215.
16. **Erlanger, B.F., Borek, F., Beiser, S.M. and Lieberman, S.** 1957. Steroid-protein conjugates. I. Preparation and characterization of conjugates of bovine serum albumin with testosterone and with cortisone. J.Biol. Chem. **228**: 713—727.
17. **Fang, S. and Liao, S.** 1969. Antagonistic action of antiandrogens on the formation of a specific dihydrotestosterone-receptor protein complex in rat ventral prostate. Mol. Pharmacol. **5**: 420—431.
18. **Fischer, L.J., Kent, T.H. and Weissinger, J.L.** 1973. Absorption of diethylstilbestrol and its glucuronide conjugate from the intestines of five- and twenty-five day-old rats. J. Pharmacol. Exp. Ther. **185**: 163—170.
19. **Fischer, L.J., Weissinger, J.L., Rickert, D.E. and Hintze, K.L.** 1976. Studies on the biological disposition of diethylstilbestrol in rats and humans. J. Toxicol. Environ. Health **1**: 587—605.
20. **Geller, J., Albert, J., Losa, D., Geller, S., Stoeltzing, W. and Vega, D.** 1978. DHT concentrations in human prostate cancer tissue. J. Clin. Endocrinol. Metab. **46**: 440—444.
21. **Ghanadian, R., Smith, C.B., Williams, G. and Chisholm, G.D.** 1977. The effect of antiandrogens and stilboestrol on the cytosol receptor in rat prostate. Br. J. Urol. **49**: 695—704.
22. **Ghanadian, R., O'Donoghue, E.P.N. and Puah, C.M.** 1979. Changes in dihydrotestosterone and testosterone following endocrine manipulation in carcinoma of the prostate. Cancer Treat. Rep. **63**: 1192.
23. **Harper, M.E., Peeling, W.B., Cowley, T., Brownsey, B.G., Phillips, M.E.A., Groom, G., Fahmy, D.R. and Griffiths, K.** 1976. Plasma steroid and protein hormone concentrations in patients with prostatic carcinoma before and during oestrogen therapy. Acta Endocrinol. **81**: 409—426.
24. **Hawkins, E.F.M., Nijs, M., Brassinne, C. and Tagnon, H.J.** 1975. Steroid receptors in the human prostate. I. Estradiol-17 β binding in benign prostatic hypertrophy. Steroids **26**: 458—470.
25. **Hawkins, R.A., Hills, A. and Freeman, B.** 1975. A simple method for the determination of oestrogen receptor concentration in breast tumors and other tissues. Clin. Chim. Acta **64**: 203—213.
26. **Hoffman, B. and Laschütza, W.** 1980. Entwickelung eines Radioimmunotests zur Bestimmung von Diäthylstilbestrol in Blutplasma und essbaren Geweben von Rind. Arch. Levensmittelhygiene. **31**: 105—111.
27. **Huber, T.L., Horn, G.W. and Beadle, R.E.** 1972. Liver and gastrointestinal metabolism of diethylstilbestrol in sheep. J. Animal Sci. **34**: 786—790.
28. **Johansson, R.** 1976. Effect of some synandrogens and antiandrogens on the conversion of testosterone to dihydrotestosterone in the cultured rat ventral prostate. Acta endocr. **81**: 398—408.
29. **Jonsson, G. and Nilsson, T.** 1976. Pharmacology of drug therapy, p. 347—353. *In* D.I. Williams and G.D. Chisholm (ed.), Scientific Foundations of Urology, vol II. Heinemann, London.
30. **Kamyab, S., Fotherby, K. and Steele, S.J.** 1969. Metabolism of 4-^{14}C-ethynyl oestradiol in women. Nature **221**: 360—361.
31. **Kemp, H.P., Reed, G.F., Riad-Fahmy, D., Pike, A.W., Gaskel, S.J., Queen, K., Harper, M.E. and Griffiths, K.** 1981. Measurements of diethylstilbestrol in plasma from patients with cancer of the prostate. Cancer Res. **41**: 4693—4697.
32. **Kliman, R.A., Mclaughlin, R.A. and Prout, G.R., Jr.** 1980. Effect of diethylstilbestrol on androgen receptors in human benign prostatic hyperplasia. 62nd Ann. Meet. Endocr. Soc. Abst. **829**: 282.
33. **Kodama, T., Honda, S. and Shimazaki, J.** 1977. Androphilic proteins in cytosols of human benign prostatic hypertrophy. Endocrinol. Jpn. **24**: 565—573.
34. **Labarca, C. and Kenneth, P.** 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. **102**: 344—352.
35. **Laschütza, W. and Hoffamn, B.** 1979. Radioimmunoassay (RIA) for detection of diethylstilbestrol (DES) in muscular tissue of calves. Acta Endocr. (Kbh), Suppl. **225**: 98.
36. **Lee, D.K.H., Young, J.C., Tamura, Y., Patterson, D.C., Bird, C.E. and Clark, A.F.** 1973. Effects of oestrogens on the Δ^4reduction of testosterone by rat prostate and liver preparation. Can. J. Biochem. **51**: 735—740.

37. **Leitem, L., Gasper, P. and Bello, I.** 1978. Stable derivatives for the gas chromatographic determination of synthetic anabolic stilbene residues (diethylstilbestrol., dienestrol and hexestrol) in meat and organs of treated cattle at the sub-parts per billion (10^9) levels. J. Chromatography. **156**: 267—273.
38. **Liao, S., Howell, D.K. and Chang, I.M.** 1974. Action of a non-steroidal antiandrogen, flutamide, on the receptor binding and nuclear retention of 5 α-dihydrotestosterone in rat ventral prostate. Endocrinology **94**: 1205—1209.
39. **Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.** 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**: 265—269.
40. **Macker, M.A., Liberti, J.P., Smith, M.J.V., Koontz, W.W., Jr. and Prout, G.R. Jr.** 1972. The effect of orchiectomy and various doses of stilbestrol on plasma testosterone levels with carcinoma of the prostate. Invest. Urol. **9**: 423—425.
41. **Meikle, A.W., Stringham, J.D. and Olsen, D.C.** 1973. Subnormal tissue 3α -androstendiol and androsterone in prostatic hyperplasia. J. Clin. Endocrinol. Metab. **47**: 909—913.
42. **Metzler, M.** 1976. Metabolic activation of carcinogenic diethylstilbestrol in rodents and humans. J. Toxicol. Environ. Health Suppl. **1**: 21—35.
43. **Miller, R.K., Heckmann, M.E. and McKenzie, C.** 1982. Diethylstilbestrol: Placental transfer, metabolism, convalent binding and fetal distribution in the wister rat. J. Pharmacol, Exp. Ther. **220**: 358—365.
44. **Mitchell, G.E., Jr., Neumann, A.L. and Draper, H.H.** 1959. Metabolism of tritium-labeled diethylstilbestrol by steers. J.Agr. Food Chem. **7**: 509—512.
45. **Morfin, R.F., Stefano, S.D., Bercovici, J.P. and Floch, H.H.** 1978. Comparison of testosterone and 5α -androstane-3α, 17β diol metabolism in human normal and hyperplastic prostate. J. Steroid Biochem. **9**: 245—252.
46. **Murphy, B.E.P.** 1968. Binding of testosterone and estradiol in plasma. Can. J. Biochem. **46**: 299—301.
47. **Oelschlager, H., Rotheley, D. and Dunzendorfer, U.** 1984. Plasmaspiegel von Fosfestrol und seinen Monophosphat, von Diethylstilbestrol und seinem Monoglucuronid nach intravenoser Gave bei Patienten mit metastasierendem Prostata karzinom. Arzneim-Forsch/Drug Res. **34**: 1333—1336.
48. **Robinson, M.R.G. and Thomas, B.S.** 1971. Effect of hormonal therapy on plasma testosterone levels in prostatic carcinoma. Brit. M.J.B.: 391—394.
49. **Rosner, W.M., Kelly, W.G., Deakins, S.M. and Christy, N.P.** 1968. The binding of estrogens and testosterone in human plasma, p. 352—340. *In* H.L. Haynes, F.A. Goswitz and B.E.P. Murphy (ed.), Radioisotopes in Medicine: In vitrol studies. Contribution, USAEC.
50. **Scatchard, G.** 1949. The attraction of proteins for small molecules and ions. Ann. NY Acad. Sci. **51**: 660—672.
51. **Schellhaas, G., Kleickmann, A. and Nitzschke, E.** 1978. Untersuchungen dünnschichtchromatographischen Nachweis von Diäthylstilbostrol im Harn von Kälbern und Jungrindern. Arch. Lebensmittelhyg. **29**: 219—222.
52. **Shah, H.C. and McLachlan, J.A.** 1976. The fate of diethylstilbestrol in the pregnant mouse. J. Pharmacol. Exp. Ther. **197**: 687—696.
53. **Shain, S.A., Boesel, R.W., Lamm, D.L. and Radwin, H.M.** 1978. Characterization of unoccupied(R) and occupied(RA) androgen binding components of the hyperplastic human prostate. Steroids **31**: 541—550.
54. **Shearer, R.J., Hendry, W.F., Sommerville, I.F. and Fergusson, J.D.** 1973. Plasma testosterone: an accurate moitor of hormone treatment in prostatic cancer. Br. J. Urol. **45**: 668—674.
55. **Sheehan, D.M. and Young, M.** 1979. Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma and human. Endocrinology **104**: 1442—1447.
56. **Scholinton, L.J., Srivastava, L. and Taylor, B.B.** 1975. The in-vitro and in-vivo effects of diethylstilbestrol on testicular synthesis of testosterone. Steroids **26**: 797—806.
57. **Smith, C.B., Ghanadian, R. and Chisholm, G.D.** 1978. Inhibition of the nuclear dihydrotestosterone receptor complex from rat ventral prostate by anti-androgens and stilboestrol. Mol. Cell. Endocrinol. **10**: 13—17.
58. **Stöckel, V.W., Bambery, E., Choi, H.S., and Götzinger, N.** 1978. Radioimmunologische Bestimmung vor Diäthylstilbestrol in Blut von Matrindern. Wien. Tierart. Mschr. **65**: 329—331.
59. **Tilley, W. and Marshall, V.R.** 1981. The management and role of androgen and oestrogen receptors in the prostate. Br. J. Urol. **53**: 107.
60. **Usui, T.** 1985. Endocrinological changes following antiandrogenic hormone therapy in patients with prostatic carcinoma. Nishinihon J. Urol. **47**: 17—28.
61. **Vogt, K.** 1980. Vergleichende Untersuchungen über den Diäthylstilbestrolgehalt in Kot, Urin und Schlachtkorpern von Kälbern mittels Dunnschichtchromatographie und Radioimmunotest. Berl. Münch. Tierarztl. Wschr. **93**: 147—149.