Acta Urol. Jap. Vol. 27
No. 3, March 1981

IN VIVO STUDIES OF ^{3}H-ESTRAMUSTINE IN CASTRATED MALE RAT

Hidetoshi YAMANAKA, Kouichi KITAURA, Kyoichi IMAI,
Hisako UASA, Katsuyuki NAKAI, Yoshio MATSUMURA,
Hisao UEHARA and Keizo SHIDA

From the Department of Urology, School of Medicine, Gunma University, Maebashi, JAPAN 371 *(Director: Prof. K. Shida, M. D.)*

Using estramustine of high specific radioactivity, we have investigated the uptake of estramustine in the different organs and some properties of binding proteins which bind estramustine and/or its metabolites in the ventral prostate of rat in *in vivo* experiments. The results and conclusions are as follows:

1. ^{3}H-radioactivity was accumulated selectively in the ventral prostate of the castrated male rat after the administration of ^{3}H-estramustine. Estramustine and its metabolites were retained in the ventral prostate for longer time, comparing with estradiol-17β or cyproterone acetate. The ammount of radioactivity in purified nucleus of the ventral prostate was only 0.39% of that in the cytosol at 24 hours after the injection of ^{3}H-estramustine. These results strongly suggested that there existed the mechanism of retention of estramustine and its metabolites in the cytosol of rat ventral prostate.

2. The sucrose density gradient analysis demonstrated that the ventral prostate cytosol binding proteins, which bind estramustine and/or its metabolites, have sedimentation coefficient of 3 to 5S and the pretreatment with injection of estradiol-17β reduced this 3–5S radioactive peak. Thin layer chromatographic analysis showed that ^{3}H-compounds which bind 3–5S macromolecules were mainly estramustine and estrone-cytostatic complex (Leo 271f) and that smaller amounts of estradiol-17β and estrone were detected. It is sure from these results that there exist in the cytosol of rat ventral prostate a macromolecule which bind hormone-cytostatic complexes and is different from androgen receptor.

In the cells which have sensitivity to a hormone, there exists a receptor that has specific affinity to this hormone. On elucidation of the mechanism of hormone -receptor interaction, investigators got an idea to utilize a hormone as a carrier of a chemotherapeutic agent. Estracyt® (estramustine phosphate) is a nitrogen mustard derivative of estradiol-17β, which is one of a series of alkylating agents synthesized in the Research Laboratory of AB Leo, Sweden and has been reported to have an excellent palliative effect on advanced prostatic cancer[1~6]. (Fig. 1) The observed effects of Estracyt in the animal experiments are similar to estrogenic effects[7~11]. But

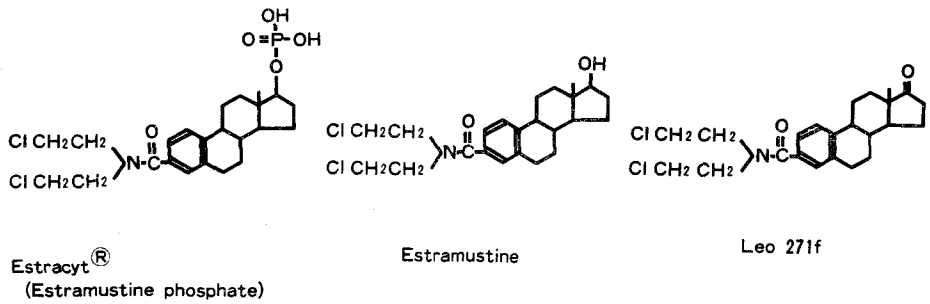

Fig. 1. The formulae for Estracyt®, estramustine and Leo 271 f.

several experimental results indicate that effect of Estracyt cannot be attributed solely to its antigonadotropic or estrogenic effect[12~16]. Plym Forshell et al[17]. demonstrated that estramustine was found to be the major metabolite in peripheral blood after oral as well as intraveneous administration. Using estramustine of high specific radioactivity, we have investigated the uptake of estramustine in the different organs and some properties of binding proteins which bind estramustine and/or its metabolites in the ventral prostate of rat in the *in vivo* experiments.

MATERIALS AND METHODS

Animals: Male adult Wistar strain rats were maintained on laboratory chow of Japan Clea Co. and water at libitum. Animals were housed in metabolic cages in a temperature-controlled room under 12 hours light ~12 hours dark lighting schedule Animals had orchiectomy via the scrotal route under ether anaesthesia 24 hours before the injection of tritiated compounds.

Chemicals: The radioactive compounds used were ^{3}H-estramustine ((6,7-^{3}H)-estradiol-17β-3N-bis-(2-chloroethyl) carbamate; 41.0 Ci/mmole), ^{3}H-estradiol-17β ((2,4,6,7-^{3}H)-estradiol-17β; 90.0 Ci/mmole), ^{3}H-cyproterone acetate (17α-(^{3}H)-acetoxy-6-chlor-1, 2α-methylen-4,6-pregnadiene-3, 20-dion; 4.2 Ci/mmole). Estramustine, estracyt (estradiol-3N-bis-(2-chloroethyl) carbamate-17β-phosphate), Leo271f (estrone-3N-bis-(2-chloroethyl) carbamate) were kindly supplied by AB Leo, Hälsingborg, Sweden. ^{3}H-cyproterone acetate was a gift from Schering AG, Berlin. ^{3}H-estradiol-17β was obtained from New England Nuclear, Boston, Mass.

Preparation of subcellular fraction: The ventral prostates were removed and homogenized with a Potter-Elvehjem homogenizer in cold 0.05M Tris-HCl buffer, pH 7.4 containing 0.25M sucrose and 1.0mM $MgCl_2$. The homogenate was centrifuged at 700$\times$g for 10 min. and then at 10000$\times$g for 15 min., to get the mitochondrial fraction. The supernatant was centrifuged at 105,000$\times$g for 60 min., to get the microsomal fraction and the cytosol fraction. The crude nuclear fraction (700$\times$g) was suspended in 2.4M sucrose–1.0mM $MgCl_2$, and then centrifuged in a Hitachi RPS 27 roter at 93700$\times$g for 40 min., to get the purified nuclear fraction.

Sucrose density gradient analysis: An equal volume of 0.25% dextran– 2.5% charcoal–10% (v/v) glycerol was added to the cytosol fraction, and then the sample was vortexed for 5 sec. and centrifuged immediately at 8,000$\times$g for 10 min., 200μl of the supernatant was layered over performed 5–20% (v/v) sucrose gradients in 50mM Tris-HCl buffer (pH 7.4)–2mM 2-mercaptoethanol–10% (v/v) glycerol. The gradients were centrifuged for 21 hours at 144,900$\times$g at 2°C in a Hitachi RPS 65T roter. Fractions (100μl each) were collected using a density gradient fractionator (ISCO, Model 640) and then dissolved in 1.0ml of distilled water, 1.0ml of ethyl alcohol and 10ml of toluene containing 0.4% PPO and 0.01% POPOP, and then vortexed for 30 sec., to extract the labelled steroid from the aqueous phase. Radioactivity was counted in a Packard Tri Carb liquid scintillation spectrometer, Model 3380, with a counting efficiency of 37~48%. ^{14}C-methylated bovine serum albumin was used as the sedimentation coefficient marker protein.

Thin layer chromatographic analysis: The radioactivity bound to macromolecules in the cytosol of ventral prostate was analyzed with thin layer chromatography. After sucrose density gradient centrifugation, the appropriate fractions were pooled and then characterized with thin layer chromatographic method, as described by P.A. Høisaeter.[18]

RESULTS

1) *The tissue distribution of ^{3}H-radioactivity after the administration of ^{3}H-estramustine and ^{3}H-estradiol-17β*

The tissue distribution of ^{3}H-radioactivity in the arious organs of the castrated male rats at the different intervals after the administration of ^{3}H-estramustine is shown in Table 1. The patterns of the tissue distribution of ^{3}H-radioactivity are the same among the examined organs excepting the ventral and dorsolateral prostates. The

Table 1. Each castrated animals received either 50 μCi ^{3}H-estramustine. of Groups ofthree animals were killed at different intervals after the intraperitoneal administration of ^{3}H-estramustine. Specimens were taken from the ventral prostate, dorsolateral prostate, liver, muscle, pancreas, kidney, pituitary gland, hypothalamus and also from serum. An appropriate amount of each tissue was dissolved in 1 ml of Soluene 350 (Packard) overnight, and then performed as pescribed in text. The results are the mean of three independent determinations.

	Radioactivity (DPM/10mg of tissue)								
hours	ventral prostate	dorsolateral prostate	liver	muscle	pancreas	kidney	pituitary gland	hypothalamus	serum*
0.5	636	320	10880	306	2297	2161	733	398	405
1	2536	533	13672	581	2137	1396	1095	617	874
2	2606	747	8085	275	782	847	558	260	711
4	3842	430	10354	300	340	683	444	173	842
6	3040	468	6880	223	405	645	357	160	699
20	2462	232	3776	120	283	439	202	87	352

* Radioactivity of serum are shown as d.p.m./0.01 ml.

ventral and dorsolateral prostates showed the increased concentration of ^{3}H-radioactivity at 2 hours after ^{3}H-estramustine administration, compared to 30 minutes. In the ventral prostate, the radioactivity uptake increased gradually and reached to maximum at 4 hours after the injection of ^{3}H-estramustine. The uptake of tracer at 20 hours was 64% of that at 4 hours. On the other hand, the maximum peak of concentration of radioisotope in the dorsolateral prostate was observed at 2 hours after the injection of ^{3}H-estramustine.

Table 2 shows the distribution of ^{3}H-radioactivity in different organs of the castrated male rat at the various intervals after the administration of ^{3}H-estradiol-17β. The same distribution pattern was observed in all organs, with a maximum concentration at 30 minutes after the injection. It is worthy to note that high and prolonged uptake of ^{3}H-radioactivity was found in the pituitary gland. In both the ventral and dorsolateral prostates, the peak concentration of ^{3}H-radioactivity was observed after 30 minutes.

These results from Table 1 and Table 2 indicate that the rat prostate, especially the ventral prostate, is capable of accumulating estramustine but not estradiol-17β.

Table 2. 50 μCi of ^{3}H-Estradiol-17 β was intraperitoneally given to each castrated rat. Groups of three animals were killed at different intervals after the administration of ^{3}H-estradiol-17 β. The following procedures were the same as those in the experiment shown in Table 1. The results are the mean of three independent determinations.

	Radioactivity (DPM/10mg of tissue)								
hours	ventral prostate	dorsolateral prostate	liver	muscle	pancreas	kidney	pituitary gland	hypothalamus	serum*
0.5	1493	590	9058	368	1083	1824	1702	435	655
1	415	411	6705	357	791	1521	1542	373	653
2	268	329	3344	281	547	871	1076	269	578
4	258	295	3421	329	438	738	1050	227	560
6	271	271	3324	295	366	608	925	180	587
20	245	252	1070	235	261	387	575	164	478

Table 3. 50 μCi of ^{3}H-compounds was intraperitoneally given to each castrated rat. The results are the mean of three independent determinations.

hours (after injection)	^{3}H-Cyproterone acetate		^{3}H-Estradiol-17β		^{3}H-Estramustine	
	ventral prostate	dorsolateral prostate	ventral prostate	dorsolateral prostate	ventral prostate	dorsolateral prostate
0.5	1.465	1.010	2.279	0.901	1.953	2.050
1	1.312	0.926	0.636	0.629	2.705	0.611
2	1.660	0.994	0.463	0.570	2.593	0.923
4	0.877	0.852	0.461	0.528	4.377	0.482
6	0.868	0.612	0.462	0.462	3.287	0.350
20	n.d*	n.d*	0.511	0.527	6.997	0.661

* not determined.

2) *The ratio between the radioactivity in the prostate and that in serum after the injection of various ^{3}H-compounds*

The ratio between the radioactivity in the prostate and that in serum at different intervals after the injection of ^{3}H-compounds to castrated rats was shown in Table 3. In the ventral prostate, the ratio after the injection of ^{3}H-estramustine was much higher, compared with that after ^{3}H-estradiol-17β or ^{3}H-cyproterone acetate injections. On the other hand, there existed no difference of the ratio among three tritiated compounds in the dorsolateral prostate.

3) *The subcellular distribution of ^{3}H-radioactivity in the ventral prostate of castrated rat*

The uptake of ^{3}H-radioactivity in the subcellular fractions of the ventral prostate was measured at the different intervals after the intraperitoneal administration of 75 μCi of ^{3}H-estramustine to castrated rats (Table 4). It was observed that the amount of radioactivity in the whole homogenates increased sharply to 6076 d.p.m. per 10 mg tissue at 4 hours and then gradually decreased to about 73% of the maximum value at 24 hours after the injection of ^{3}H-estramustine. The radioactivity in the cytosol fraction also reached a maximum value of approximately 4000 d.p.m. per 10 mg tissue at 4 hours, and then slightly decreased to about 90% of the 4 hours value at 24 hours after the injection of ^{3}H-estramustine.

The result from this Table indicates that estramustine and/or its metabolites are retained only in the cytosol of the ventral prostate for a long time after the injection of this compound.

4) *Analysis of ^{3}H-radioactivity in the cytosol of ventral prostqte following labelling in vivo*

Fig. 2 shows estramustine and its metabolites bound to macromolecules from the cytosol of ventral prostate of castrated rats

Table 4. Groups of five rats castrated 24 hours previously were injected with 75 μCi of ^{3}H-estramustine. Then 0.5, 1, 4, 24 hours later, the rats were killed and the subcellular fractions were prepared as described in text. The results are the mean of three independent determinations.

Subcellular fraction	Radioactivity (DPM/10mg tissue) 0.5 hr	1 hr	4 hr	24 hr
whole homogenates	2321	2590	6076	4481
cytosol	1290	1473	3954	3592
crude nuclei	707	1125	1371	495
purified nuclei (2.4Msucrose)	15	13	26	14
mitochondria	227	174	286	83
microsome	78	64	116	100

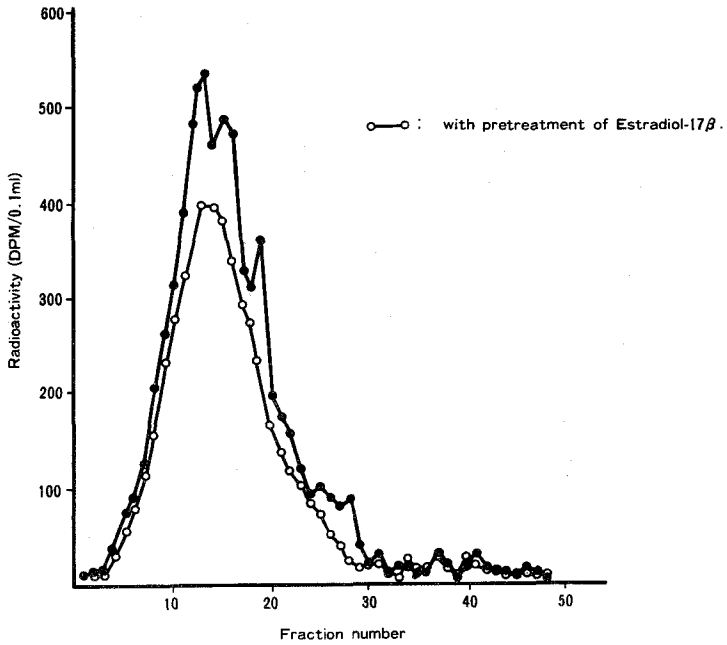

Fig. 2. The 24 hours previously castrated rats were divided into two groups (the control group and the estradiol-17 β treated group) Each group consisted of 5 rats. The estradiol-17 β treated group were injected intraperitoneally with 500 μg of estradiol-17 β and the control group rats with the vehicle. 30 min. later, the animals were injected intraperitoneally with 90 μCi of ^{3}H-Estramustine. At 60 min. after the second injection, the rats were killed and the cytosol fraction was prepared as described under "MATERIALS AND METHODS". After treatment with dextran-charcoal solution, 200 μl (protein, 2.16 mg) charcoal resistant supernatant were layered on sucrose gradients and sedimented at 144900$\times$g for 21 hrs at 2°C in Hitachi RPS 65T rotor.

in a linear 5$\sim$20% sucrose gradients with or without pretreatment of estradiol-17β. A prominent peak of ^{3}H-radioactivity was observed at 3–5S regions. It was also observed that 3–5S radioactive peak was reduced about 20% with the pretreatment of estradiol-17β. A prominent peak of ^{3}H-radioactivity (Fraction No.8$\sim$No.23) from the cytosol of the ventral prostate without pretreatment of estradiol-17β was pooled and analyzed by thin layer chromatography (Fig. 3). Two major spots were those which correspond to estramustine and estramustine-congener, Leo 271, respectively. The smaller radioactivities had mobilities corresponding to those of estradiol-17β and estrone.

DISCUSSION

According to the present results, ^{3}H-radioactivity was accumulated selectively in the ventral prostate of the castrated male rat after the administration of ^{3}H-estramustine. It was also demonstrated that estramustine and its metabolites were retained in the ventral prostate for longer time, comparing with estradiol-17β or cyproterone acetate. It was strongly suggested from these results that there existed the mechanism of retention of estramustine and its metabolites in the cytosol of rat ventral prostate. In fact, Høisaeter[19] has observed that the ventral prostatic cytosol contained macromolecules which have higher affinity towards the hormone-cyto-

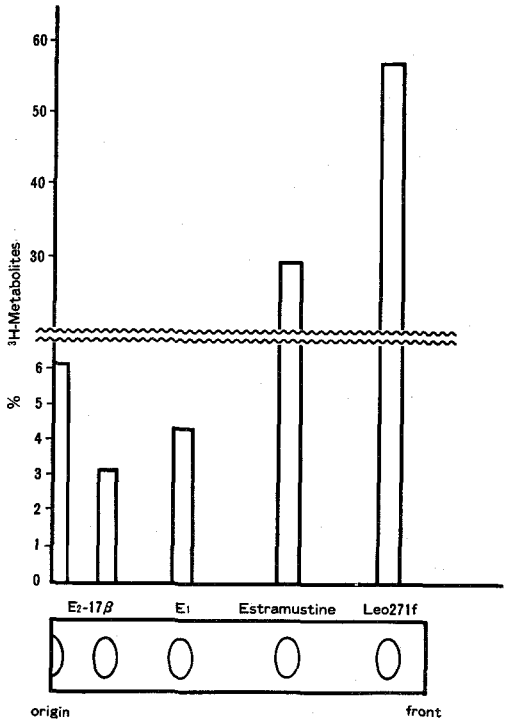

Fig. 3. Chromatography of ethyl acetate extracts from the pooled fractions after suerose density gradient centrifugation was perfo-rmed with chloroform as the solvent phase. The positions of the reference standards on the TLC plate are shown below. Rf. of estradiol-17 β, estrone, estramustine and Leo 271 f are 0.145, 0.352, 0.630 and 0.903, respectively.

static complexes than have macromolecules in rat liver or muscle cytosol. According to the report of Forsgren et al.[20], a estramustine binding protein had a molecular weight of 40,000 to 50,000, a Stokes' radius of 2.9 nm, a fractional ratio of 1.2, a sedimentation coefficient of 3.5 to 4S, and an isoelectric point of 5. It has been already reported by several investigators that there exist a macromolecule in rat ventral prostatic cytosol, the properties of which is similar to a estramustine binding protein. Liao et al.[21] designated this protein as "α-protein" and Heynes et al.[22] and Lea et al.[23] did it as "prostate binding protein" and "prostatein" respectively.

Høisaeter[19] reported that after incubation of minced ventral prostate tissue with ^{3}H-estramustine, radioactivity was recovered from the nuclear preparations. But, our present results in the *in vivo* experiment (Table 4) showed that the amount of radioactivity in purified nucleus was only 0.39% of that in the cytosol 24 hours after the injection and that ^{3}H-radioactivity in the nucleus was not retained in course of time, while the retention of ^{3}H-radioactivity in the cytosol of ventral prostate was observed. The sucrose gradient and thin layer chromatographic analysis demonstrated (Fig. 2) that pretreatment with estradiol-17β reduced 3–5S radioactive peak One possible explanation for this observation is that estramustine and/or its metabolites in part are bound to the same binding site of estradiol-17β in the prostate cytosol. In fact, it was demonstrated in the present study that ^{3}H-compounds that bind charcoal resistant cytosol protein were mainly estramustine and Leo 271f and that smaller amounts of estradiol-17β and estrone were detected. Kirdani et al.[24] observed the decreased uptake of labelled estradiol and estriol into the prostate of the dog treated with estramustine phosphate and concluded that these phenomena could have resulted from competition of unlabelled estrogens produced by a central hydrolysis of the compound.

It is sure from our present and other investigator's results that there exists in the cytosol of rat ventral prostate a macromolecule which bind hormone-cytostatic complexes and is different from androgen receptor.

Further studies for the participation of this macromolecule in the intracellular regulation of steroid receptor complex in the cytosol of the prostate are ardently awaited.

ACKNOWLEDGEMENTS

This work was partly supported by a Grant from the Ministry of Education, Science and Culture, Japan and a Grant-in Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

REFERENCES

1) Andersson, L., Edsmer, F., Jönsson, G. and Könyves, I.: Estramustine phosphate therapy

carcinoma of the prostate. Internat. Symp. Dusseldorf pp.24～25, 1975.
2) Jönsson, G., Högberg, B., and Nilsson, T.: Treatment of advnced prostatic carcinoma with estramustine phosphate (Estracyt). Scand. J. Urol. Nephrol., **11** : 231, 1977.
3) Szendröi, Z., Könyves, I., Szendi, L., Eckhard, S., and Hartay, F.: Estracyt in hormone-resistant prostatic carcinoma. Internat. Urol. Nephrol., **6** : 101, 1974.
4) Mittelman, A., Shukla, S. K. and Murphy, G. P.: Extended therapy of stage D carcinoma of the prostate with oral estramuxtine phosphate. J. Urol., **115** : 409, 1976. s
5) Benson, R. C., Wear, J. B. and Gill, G. M.: Treatment of stage D hormone-resistant carcinoma of the prostate with estramustine phosphate J. Urol., **121** : 452, 1979.
6) Estracyt Research Group (chairman: Takayasu, H., co-chairman: Shida, K.): Clinical study on the treatment of prostatic carcinoma with Estracyt. The Nishinihon J. Urol., **42** : 715, 1980.
7) Fosså, S. D., Fosså, J. and Aakvaak, A.: Hormone changes in patients with prostatic carcinoma during treatment with estramustine phosphate. J. Urol., **118** : 1013, 1977.
8) Gustafson, A., Nilsson, S., Persson, B., Tisell, L. E., Wiklund, O. and Ohlson, R.: Treatment with estramustine phosphate (Estracyt) in prostatic carcinoma. Invest. Urol., **15** : 220, 1977.
9) Yamanaka, H., Shimazaki, J., Imai, K., Sugiyama, Y. and Shida, K.: Effects of Estracyt on the rat prostate. Invest. Urol., **14** : 400, 1977.
10) McMillin, J. M., Seal, U. S. and Doe, R. P.: Effect of oral estramustine phosphate on piyuitary, gonadal, and adrenal function in the green monkey (Cercopithecus aethiops sabaeus). Invest. Urol., **15** : 151, 1977.
11) Kjaer, T. B., Nilsson, T. and Madsen, P. O.: Effect of estramustine phosphate on plasma testosterone during treatment of carcinoma of prostate. Urology, **5** : 802, 1975.
12) Høisaeter, P. A.: Incorporation of ^{3}H-thymidine into rat ventral prostate in organ culture: Influence of hormone-cytostatic complexes. Invest. Urol., **12** : 479, 1975.
13) Høisaeter, P. A.: Incorporation of ^{3}H-thymidine and ^{14}C-amino acids into the ventral prostate after in vivo treatment with estradiol-3N-bis (2-chloroethyl) carbamate-17β-phosphate (Estracyt®) and its estrogenic and cytostatic parts. Invest. Urol., **14** : 85, 1976.
14) Müntzing, J., Kirdani, R. Y., Saroff, J., Murphy G. P. and Sandberg, A. A.: Inhibitory effects of Estracyt on R-3327 rat prostatic carcinoma. Urology, **10** : 439, 1977.
15) Smolev, J. K., Heston, W. D. W., Scott, W. W. and Coffey, D. S.: Characterization of the Dunning R3327H prostatic adenocarcinoma: an appropriate animal model for prostatic cancer. Cancer Treat. Rep., **61** : 273, 1977.
16) Yamanaka, H., Koya, A., Imai, K., Nakai, K., Yuasa, H., Sugiyama, Y., Matsumura, Y., Uehara, H., Saruki, K., Shida, K. and Wakabayashi, K.: Fundamental investigation of Estradiol-3N-Bis-(2-chloroethyl) carbamate-17β -phosphate (Estracyt®) — the antiprostatic effect of this drug — Clinical Endocrinology, **29** : 1981 (in press).
17) Plym Forshell, G., Müntzing, J., Ek, A., Lindstedt, E. and Dencker, H.: The absorption, metabolism, and Extretion of Estracyt (NSC 89199) in patients with prostatic cancer. Invest. Urol., **14** : 128, 1976.
18) Høisaeter, P. A.: Studies on the conversion of oestradiol linked to a cytostatic agent (Estracyt®) in various rat tissues. Acta Endocrinol., **82** : 661, 1976.
19) Høisaeter, P. A.: In vitro binding of tritiated hormone-cytostatic complexes in the cytosol of various rat tissues and the incorporation of these complexes into nuclei. Scand. J. Urol. Nephrol., **11** : 135, 1977.
20) Forsgren, B., Hogberg, B., Gustafsson, J.-Å. and Pousette, Å.: Binding of estramustine, a nitrogen mustard derivative of estradiol-17β, in cytosol from rat ventral prostate. Acta Pharm. Suec., **15** : 23, 1978.
21) Liao, S., Tymoczko, L., Castanedo, E. and Liang, T.: Androgen receptors and androgen -dependent initiation of protein synthesis in the prostate. Vitamins and Hormones, **33** : 297, 1975.
22) Heyns, W. and De Moor, P.: Prostatic binding protein — a steroid binding protein secreted by rat prostate. Eur. J. Biochem., **78** : 221, 1977.
23) Lea, O. A., Petrusz, P. and French, F. S.: Isolation and characterization of prostatein: a major secretory protein of rat ventral prostate. (Abstract) Fed. Proc., **36** : 780, 1977.
24) Kirdani, R. Y., Muntzing, J., Varkarakis, M.J. Murphy, G. P. and Sandberg, A. A.: Studies on the antiprostatic action of Estracyt, a nitrogen mustard of estradiol. Cancer Res., **34** : 1031, 1974.

(Accepted for rapid publication, November 17, 1980)

和文抄録

去勢雄性ラットにおける ^{3}H-Estramustine *In vivo* 実験

群馬大学医学部泌尿器科学教室（主任：志田圭三教授）
山中英寿・北浦宏一・今井強一
湯浅久子・中井克幸・松村嘉夫
上原尚夫・志田圭三

Estradiol-3N-Bis-(2-chloroethyl) carbamate-17β-phosphate (estramustine phosphate, Estracyt®) はホルモンを担体として制癌剤を高濃度に目的とする癌組織に運び，そこで制癌効果を発揮させようという概念のもとに開発されたエストロゲン一制癌剤複合体であり，進行性前立腺癌の治療薬としてその有効性が報告されているが，今回，高比放射能活性をもつ ^{3}H-estramustine を用いて去勢ラットにおける本薬剤の生体内動態について，特に前立腺に焦点をあわせて検討し，以下のような結果および結論が得られた．

1．去勢雄性ラットに ^{3}H-estramustine を腹腔内投与し，各臓器における放射活性を検討したところ，前立腺腹葉への ^{3}H-放射活性の選択的なとり込みが観察された．このような現象は ^{3}H-estradiol-17β および ^{3}H-cyproterone acetate 投与の際には観察されなかった．さらに ^{3}H-放射活性の前立腺腹葉における細胞内分布を経時的に追求したところ，精製核分画へのとり込みはサイトゾール分画の1.2～0.4%にすぎず，^{3}H-放射活性のとり込みの大部分はサイトゾール分画にみられた．これらの結果より前立腺腹葉サイトゾール分画には estramustine およびその代謝物を長時間に保持する機構が存在することが示唆された．

2．前立腺腹葉サイトゾール分画に存在する estramustine またはその代謝産物の結合蛋白に沈降常数は3～5 S であり，これら結合蛋白はアンドロゲンリセプターとは異なる蛋白であることが推測された．