CHAPTER 1

INTRODUCTION

It has been reported by the National Cancer Institute, Thailand, published in Cancer Statistic, 1978 (1) concerning the 10 leading sites of cancer in Thai female that breast is the primary site of 13.12% of all cancers diagnosed in 2,416 women. Breast cencer incidence is second only to cancer of the cervix uteri (30.46%). Our studies indicate that mastectomy is the most common practice for these patients. After surgery, about 20% of the patients were further treated by radiation only, and 10% by radiation and chemotherapy. Nearly 30% of the patients were subjected to combined treatment previously mentioned including endocrine therapy. The term "endocrine therapy" used in this thesis includes the ablative endocrine therapy; such as bilateral oophorectomy and hypophysectomy, as well as the use of antiestrogen compounds; such as diethylstilboestrol (DES) (2) and tamoxifen (TAM) trans 1-(4-β-dimethylaminoethoxyphenyl)-1, 2-diphenyl but-1-ene, Nolvadex; Imperial Chemical Industries Ltd. (3).

As early as 1896, Beatson (4) reported that bilateral oophorectomy in premenopausal women with metastatic breast cancer resulted in regression of the lesions. This type of tumor was therefore recognized as "hormone dependent" because their growth

was influenced by fluctuation in the levels of steroid sex hormones as they undergo regression after surgical removal of glands responsible for production of supporting hormones. However not until 1952 started the promising era of ablative endocrine therapy for advanced breast cancer, begining with Huggins and Bergenstal (5) reporting that bilateral adrenalectomy caused significant remission in postmenopausal women, followed by Luft and Olivercona (6) that observed similar remission after hypophysectomy.

Unfortunately, not all the breast cancer patients respond to ablative endocrine therapy. More than 50% of the premenopausal patients and even more of the postmenopausal ones do not respond to endocrine ablation (7). In 1961, Folca et al (8) indentified estrogen receptor protein (ER) in the cytosol of human breast cancer tissue. Jensen et al in 1971 (9) observed a striking correlation between the presence of ER in advance breast cancer and treatment by adrenalectomy. Increasing successful endocrine treatment to patients whose tumors were ER positive (ER^+) were reported (10, 11, 12). The responsive rate varies from 30% in general breast cancer to 60% in ER rich tumors. Patients with receptor poor tumor rarely response to hormone therapy. The latter class can thus be spared from unnecessary endocrine ablative operation (10, 11). It is noteworthy though that not all ER^+ tumors respond to endocrine treatment and this has led to the concept that ER alone is not sufficient as marker to identify hormone dependency.

Since it is well established in animal models that cytosol estrogen receptor protein (ER) and progesterone receptor protein (PgR) are among the final translated products of gene expression induced by the trigger of estrogen nuclear receptor complex (13), The presense of both ER and PgR at sufficiently high concentrations should indicated the through-action of estrogen in that target cells.

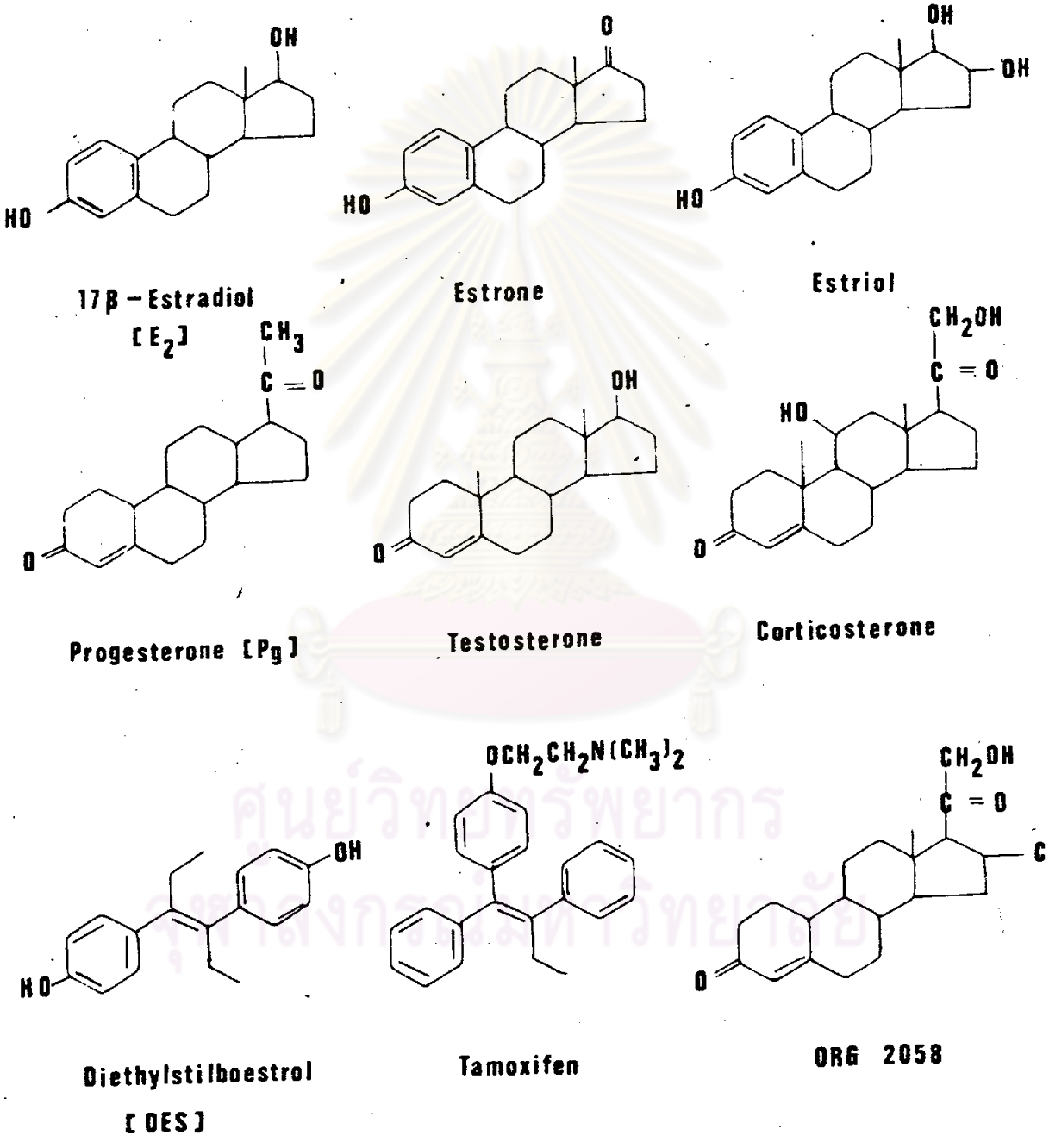
In 1975, Horwitz and McGuire have demonstrated PgR in human breast cancer (14) and have proposed that PgR, might serve as another marker of estrogen action in breast cancer (15). Horwitz and McGuire (16) explain that cancerous tumors which contain ER and PgR, may indicate that ER remains responsive to estradiol and still control specific protein synthesis. Determination of both ER and PgR in human mammary tumor as reported by McGuire in 1977 (17) showing that from the total human breast cancer specimens, 75% were ER^+. Among these ER^+ samples, 74% of them also contained PgR, whereas only 9% of the ER^- samples ccntained PgR. The responsive rate of the tumor with both receptors (ER^+ PgR^+) to endocrine therapy was doubled to 81% when the tumor with ER alone was 41% (17). This figure fits well with his report in 1975 that 40% of the observed ER^+ tumor where PgR was not determined at that time failed to respond to hormone therapy or endocrine ablation (11).

As for the biochemical characters of these two steroid hormone receptors, Wittliff et al (18) and Gardner and Wittiff (19)

have studied cytoplasmic ER from human breast carcinoma and lactating mammary glands of the rat. They reported that the sedimentation coefficient of ER was about 8 - 9S in 5 - 20% sucrose density gradient. These cytosolic estrogen receptors are specific for estrogen as judged by competitive binding studies, and demonstrate exceptionally high affinity for 17-β estradiol (Kd = 10^{-9} M) (18, 19) McGuire (20) found 8S and 4S peads by sucrose gradient centrifugation (SGC) analysis in which the 4S receptor bound nonspecificly to 17-β estradiol. The concentration of ER in both primary and metastatic mammary carcinoma varied over a wide range from 0 to 628 fmol/mg protein.

ER has been purified from calf uterus by Sica et al in 1977 (21) and from human uterus by Coffer et al (22) in the year by using affinity chromatography. The steroid binding specificity studies of pure receptor by Puca in 1979 (23) reveal that only estrogenic molecules are effective in displacing the bound $^{3}H\text{-}E_2$, estradiol being the best displacer followed by estrone and estriol (Figure 1). The purified ER as well as the native crude receptor sediments mostly at 8S with a shoulder at about 4S when analyzed on 5 - 20% sucrose gradient in low salt buffer, and dramatically changes to a 4 to 5S peak with increasing salt concentration (0.4 M KCl) in the buffer. The apparent molecular weight (mol. wt.) of ER in SDS gel electrophoresis is 70,000 daltons, and there is only one binding site for estrogen per

Figure 1. The structure of steroid hormones and substances involved in this study



70,000 mol. wt. receptor unit. The most important property of ER is the tendency of receptor to form large and irreversible aggregates even after complete purification.

In the case of PgR, Horwitz and McGuire (14) have demonstrated in human breast tumor the presence of specific PgR which sediments at 8S in SGC analysis. The dissociation constant (Kd) obtained by Scatchard analysis were approximately 2×10^{-9} M, which indicated high affinity of this receptor.

The concept of cytosolic ER also introduced to clinical usage, the potential of antiestrogens, such as DES and TAM for the treatment of mammary carcinoma.

The purpose of this investigation, partially is to fulfill the main research project "A Study on Receptor Protein of Some Steroid Hormones in Human Breast Cancer" by determining the PgR content and its Kd in the breast tumor specimens collected and previously determined for ER by Parinayakosol and Boonjawat (24). The comparative study of Kd in malignant and benign tumors will be done by Scatchard analysis using dextran-coated charcoal (DCC) assay. Distribution of PgR and ER in a population of breast tumor specimens in Thailand will be determined in order to assess the propability of hormone dependence in this population.

Some biochemical properties of PgR and ER namely the apparent sedimentation coefficient, and the molecular species responsible for

specific binding with ^{3}H-Pg and ^{3}H-E_2 will be studied by SGC analysis. The specific binding of ER and PgR, in vitro, with some steroid hormones, such as estradiol or progesterone, itself, and testosterone, corticosterone, and some synthetic antiestrogen compounds, such as DES and TAM will be studied by competitive binding assay based on DCC method. These compounds of interest either show structure (Figure 1) or function related to the action of steroid hormones in the socalled hormone dependent breast tumors. Thus, their effect on the ER binding character in vitro may throw some light on the understanding of breast tumor incidences in this country.