CHAPTER IV

DISCUSSION

The determination of ER in breast tumor cytosol has been and extensively to predict the response of breast tumor to endocrine therapy (7, 10). However 40% of tumor with measurable ER fail to respond to endocrine manipulation (15). A better indication of hormone dependency was therefore suggested recently by the additional assay of PgR (14). The presense of PgR in the cytosol of human breast tumor indicated that the tumor cells were exposed to and were capable of responding to circulating estrogen (14). PgR is therefore assayed and found to increase the clinical predictability of ER^+ patient towards endocrine therapy from 60 to 80% (15, 17, 33). McGruire, (17) had reported that 74% ER^+ tumor also contained PgR, and these designated ER^+ PgR^+ patients responded to endocrine therapy as high as 81%. In our study we have found 80 samples that contained ER, in which 64 samples or 80% (Table 1) also contained PgR. Hence the distribution of PgR^+ in the ER^+ group of Thai patients is more or less similar to that reported previously, but in the ER^- group we found PgR 31/64 (77.5%) instead of 12/129 (9%) as reported by Mcquire (17). The reason is Mcquire used ^3H - R 5020 (17, 21 dimethyl-19-nor-4, 9-pregnandione-3; 20-dione), the synthetic progestin whose binding specitiicity is restricted to progesterone receptor, while we used ^3H - Pg.

Horwitz and McGuire, 1975 (14) have reported that PgR in human breast cancer has high affinity showing average Kd = 2×10^{-9}M. In this investigation the PgR found in 90/120 specimens obtained from several hospitals in Bangkok had the average Kd = 8.58×10^{-9}M with expanding range of progesterone binding content from $0.12 - 28.23 \times 10^{-9}$M. In 1978, McGuire et al. (34) also reported that PgR in the MCF-7 human breast cancer cell line in culture was ranged from 50 - 100 fmol/mg protein which was the basal level. Raynaud and Moguilewskgy (35) suggested that great deviation of PgR content in the binding assay was caused by contamination of plasma proteins; such as glucocorticoid receptor and corticoid binding globulin that could also bind with progesterone, and resulting in apparently high content of PgR.

Comparative study of ER and PgR distribution in malignant and benign tumors were shown in Table 2 and 3. Our results showed that IDC was the most common type of breast carrinoma found in Thai women, and the proportion of "hormone dependent type" of either malignant or benign tumors as evidenced by ER^+ PgR^+ status was approximately 50%. Our results showed that fibroadenoma were found with ER and PgR together more than fibrocystic disease and gynaecomastia. Several authors (28, 36, 37) reported that high proportion of human breast carcinoma contain specific, high affinity ER which were not detected in normal breast tissue, and in the majority of benign tumors. However in a certain number of benign tumors that ER and PgR were detected, no concensus was drawn. Grilli et al. (38) reported the finding of receptors more frequently in cystic breast disease than mormal,

fibroadenoma and gynaecomastia. Leclercq et al. (37) also reported that in hyperplastic tissue (benign:fibroadenoma, papilloma) contained receptor whereas the normal female mammary gland did not. There were only 29 samples of benign tumors available in our studies, and remarkably, some male gynaecomastia, which may also be regarded as hyperplastic breast tissue contained receptors which resembled the finding of Leclercq (37). Feherty et al. (36) suggested from his results that no influence of histological feature was found to be involved in the variation of receptor concentration. In our results, both malignant and benign types of tumors showed a wide range in concentration of both receptors, where PgR showed significantly higher average content in both types but significantly lower affinity than the ER (Table 4). These finding could be explained by the limited amount of tissue obtained from each specimen, which are most frequently not sufficient to perform another set of assay using 100 fold of unlabelled material, to subtract the value of non specific binding. The apparently high content of the less specific PgR could result from the summation of enormously nonspecific binding plus minute amount of specific binding as indicated by the decreasing affinity shown in the Kd value.

The distribution of ER adn PgR in various age group were shown in Fig 2, this study implied that PgR and ER were more likely to be found at higher percentage in the postmenopausal (> 50 yr) than the premenopausal women. These results confirmed the report of Hähnel et al. (39) and Roberts et al. (40) that the ER^+ status in postmenopausal patients was higher than in premenopausal patients.

Maass et al. (41) found that in premenopausal patients, if the concentration of estradiol circulating in the plasma was over 300 pg/ml, the ER binding capacity in tissue could not be detected. Thus the high concentration of endogenecus estradiol, if present could result in the false negative of the binding assay.

In the sedimentation analysis of the cytosol receptors in some breast tumor specimens, we have found that 17 β - ^{3}H - E_2 exhibited 2 binding molecular species of the sedimentation coefficient 8 - 9S and 4S in sucrose gradient as shown in Fig 3A, B, C and D. This finding was similar to sereral reports namely Toft and Gorski(42), Wittliff et al. (18) and Gardner et al. (19) using cytosolic ER from rat uterus, human breast cancer and rat lactating mammary gland respectively. These anthors reported that cytosolic ER showed mostly 8 - 9S and also 4S in low salt gradient, Gardner et al. (19) reported that in high salt (0.4M KCl) gradient. McGuire (20) found that human mammary carcinoma contained both 8S and 4S but the 4S peak showed lower specificity in ^{3}H - E_2 binding. Purified ER from calf uteri mostly showed 8S peak with a shoulder at 4S in low salt sucrose gradient, but only one peak at 4S in high salt (23). Pavlix and Rutledge (43) found that the aggregated 8S form of cytosol ER changed to 4S monomeric form on sucrose gradient when contaminated with 0.1% of Triton X - 100. Alternatively Wittliff (44) found 4S form of ER in human breast tumor displayed in low ionic strenght sucrose gradient in a number of patients that did not response to hormone therapy. In this investigation, at

least one sample showed only 4S peak (Fig 3 B) but no information was available for response to endocrine therapy.

The sedimentation coefficient of PgR as shown in this study was consistently about 4S (Fig 4). Althought Horwitz and McGuire (14) reported both 4S and 8S peak in the human breast cancer, Allen and Leavitt (45) found that, in rat the PgR of vaginal and uterine tissue showed the sedimentation coefficient of 6 - 7S at low ionic strenght.

It can be concluded from our studies that in 120 hreast tumor specimens investigated, about 50% can be classified as hormone dependent. This is evidenced by the capability of these cytosols to bind estradiol and progesterone at high affinity as determined by DCC method. Our results obtained from the SGC analysis demonstrated that molecular species responsible for the specific binding of estradiol, designated estrogen receptor displayed the sedimentation coefficient at 8 - 9S and 4S which resembled the finding of many other investigators. Similarly, the results confirms that the molecular species responsible for specific binding of progesterone in these tumors are of the 4S species.

In the competitive binding study of estrogen receptor, tamoxifen is one of the interesting material tested, because it was a synthetic compound that antagonize the effect of estrogen on its target tissue, and currently used in the treatment of human breast cancer (3, 34). Capony and Rochefort (46) and Rochefort et al. (47) reported that tamoxifen in vitro binds directly with high affinity to the 8S ER with 200 - 300 fold less efficient comparing to estradiol itself. In our results

(Fig 5, 6, 7) tamoxifen *in vitro* at 100 fold concentration can not compete for ER binding in the 4 samples investigated. Sutherland *et al.* (48) showed that tamoxifen could bind to ER and translocated into the nucleus, but didn't showed cytoplasmic ER replenisment. Further study of Sutherland *et al.* (49) using ^{3}H - tamoxifen exhibited that only 5 - 7% of administered tamoxifen was bound to the ER in the mammary carcinoma cytosol. These results rendered the possibility that tamoxifen could have another natural ligand that mediated the antagonistic effect of the drug, whereas the agonistic effect was mediated through the ER. In our cases therefore, it might be possible that tamoxifen exerted its effect mainly as an antagonist.

DES was another synthetic estrogenic compound currently used in the treatment of human breast cancer. Our results in Fig. 6 and 7 showed that, it effectively competed for ER binding in at least 3 cytosols investigated. These results supported Funder (2) who found that both high and low salt concentration of uterine cytosol from immature rat had a high affinity of binding to DES, judged by their ability to compete with ^{3}H - E_2 for ER binding site.

The androgen is known to have a specific binding site on the ER at which the binding causes receptor translocation into the nucleus (50), but Heise and Gorlich (51) found that physiological concentration of testosterone propionate had no effect on the growth pattern of DMBA induced tumor. In our results testosterone *in vitro* did not compete for the ER and PgR binding as shown in Fig 6, 7 and 8.

As for the specific binding study of PgR, Darnell Bauer and Gorell (52) found that progesterone effectively competed for the ovine uterine PgR binding, while DES, testosterone failed to compete. The same phenomena was observed in our results as shown in Fig 8A except that the per cent displacement was very low. It is possible that this cytosol was contaminated with plasma proteins. Since it is known that progesterone has high affinity for glucocorticoid receptor present in th the plasma, if contaminated in some mammalian breast cancer (35). When ^{3}H - ORG 2058, the synthetic progesterone was used in the assay (Fig 8B). It is clearly shown that ORG 2058 obviously allows more specific measurment of PgR without interference from other steroids.

In any bioassay, it is necessary to know for how long and in what conditions the material can be stored to give accurate results Gardner and Wittliff (19) found that the ER receptor is stabilized when complexed with its ligand at 25 ^{o}C for 2 h. Sodium molybdate was reported useful for the stabilization of the 8 - 9S ER in SGC analysis (53). When the cytosols were stored in separated aliquots at -86 ^{o}C and were reexamined at intervals up to 2 months, no significant loss in estrogen binding capacity were observed (18). In this study the stability of PgR in the cytosol, stored in seperated aliquots at -70 ^{o}C was tested at various time intervals up to 3 months. Athough the number of investigations was very limited, the results shown in Table 5 suggested that the cytosol prepared for PgR assay might be stored in this condition for 2 month with the per cent of variation in Kd values less than 15%.

The presence of glycerol (10%) in the assay buffer (52) and in the sucrose density gradient (3 - 8%) was observed to stabilized the PgR during the binding study and SGC analysis (13).

All these findings indicate that, in the future PgR assay should be conducted within 2 months after sample collection, and under all precautions previouly mentioned.