

## DI-Iodothyronine as Part of the Oestradiol and Catechol Oestrogen Receptor — The Role of Iodine, Thyroid Hormones and Melatonin in the Aetiology of Breast Cancer

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**Abstract** — Hypothyroidism and low iodine intake may be important aetiological factors in oestrogen dependent tumours of the breast, uterus and ovary. They are preventable risk factors. Iodine supplementation will hopefully lead to a decreased incidence of these cancers in future generations.

The present author proposes that the tyrosyl residue in the hydrophobic oestrogen binding site of the oestrogen receptor is post translationally modified to monoiodotyrosine and hence 3,3' di-iodothyronine monoamine ( $T_2$ ) by peroxidase activity. He has previously proposed that various monoamine receptors are also  $T_2$  based. The densities of these receptors are increased in hypothyroidism and they exert control over release of prolactin and other hormones, including melatonin at multiple sites in the hypothalamic — pituitary axis. Melatonin is a metabolite of serotonin and hence melatonin receptors may be  $T_2$  or  $rT_3$  based as well. These factors could be significant in the aetiology of breast cancer as high prolactin and melatonin levels may be protective.

Oestrogen receptor density may be increased in hypothyroidism as is certain monoamine receptor density. This would amplify the effect of high circulation oestrogen levels in hypothyroidism and may help explain why hypothyroidism and low iodine intake are risk factors for breast, uterine and ovarian cancer.

### Introduction

Subtle defects in thyroid function are a feature of breast cancer and do exert a minor but not negligible effect on the incidence and clinical course of breast cancer (1). The role of oestrogen as an aetiological factor in breast, uterine and ovarian cancer is established. In 1896

Beatson pioneered the use of oophorectomy to induce tumour regressions in patients with advanced breast cancer. (2). In the same paper he advocated the use of thyroid extract as adjuvant therapy. Recently there has been considerable interest in the possible role of iodine and thyroid hormones in the aetiology and prognosis

of breast cancer and other oestrogen dependant tumours (1). The results of most studies have been equivocal. Demographic surveys however show substantive evidence of a link between breast cancer and iodine deficiency. Breast cancer mortality rates are higher in areas of endemic goitre in the U.S.A. (3, 4). The areas around Graz in Southern Austria have a ten fold increase in incidence of breast cancer compared to other countries. The population of the area was almost totally afflicted with goitre until iodine supplementation was introduced (5). The incidence and mortality rate of breast cancer is low in countries where endemic goitre is rarely found (Chile, Japan, Iceland). The reverse is true in countries where thyroid diseases are quite commonplace (1).

Stadel observed that geographical differences in the incidence of breast, ovarian and endometrial carcinoma were inversely correlated with iodine intake (6). Low iodine intake leads to a hyperoestrogenic state. Stadel's theory has been extended by Siiteri et al (7) who showed that hypothyroidism is associated with up to 80–90% free oestradiol levels, against a normal value of 40–60%. Hyperthyroidism is associated with only 20% free oestradiol levels. These differences are due to thyroid hormone induced alterations in the sex hormone binding globulin (SHBG) levels.

It has not yet been resolved whether the increased incidence of oestrogen dependant tumours in patients with abnormal thyroid function is due to a primary thyroid defect or to a secondary defect in hypothalamic — pituitary function also causing other endocrine changes (1). The present author's proposal that  $T_2$  is incorporated into the ligand binding sites of oestrogen receptors, as well as  $\alpha_1$  adrenergic,  $D_2$  dopaminergic, and certain serotonergic receptors implies that both the above factors are important. Oestrogen receptor synthesis may be influenced by thyroid status, just as the synthesis of the monoaminergic receptors is. The monoaminergic receptors in turn control the release of other hormones such as prolactin and melatonin which may also be important in the genesis of oestrogen dependant cancers. Thyroid hormones have accurately been described as permissive hormones, in that their presence is required for other hormones to be able to express their effects (8). The present author believes this to be due to the incorporation of various thyroid hormones into catecholamine,

serotonin, melatonin, opiate and oestrogen receptor proteins.

#### *The effect of a low iodine diet on the hypothalamic — pituitary axis*

The effect of hypothyroidism and a low iodine diet on monoamine receptors has been well studied (9, 10, 11, 12). In most tissues hypothyroidism leads to an increase in  $\alpha_1$  adrenergic (9, 10),  $D_2$  dopaminergic (11) and certain serotonergic receptor densities (12). The present author has previously proposed that these receptors are 3,3' di-iodothyronine ( $T_2$ ) based (13). If oestrogen receptors are also  $T_2$  based they may also increase density in hypothyroidism. Hyperthyroidism is, on the other hand, associated with an increase in beta adrenergic receptor density and the present author has previously proposed that beta $_1$  adrenergic receptors are tri-iodothyronine ( $T_3$ ) based (13).

Thyroid releasing hormone (TRH) is under a dual monoaminergic control system with a positive dopaminergic and noradrenergic and a negative serotonergic input. TRH in turn stimulates thyroid stimulating hormone (TSH) and prolactin release by two independent mechanisms (1). In addition dopaminergic stimulation of  $D_2$  dopaminergic receptors inhibits prolactin and melatonin release. Therefore alterations in thyroid status can be expected to have complex effects on prolactin secretion and secretion of other hormones for the reasons given above. Blockade of  $D_2$  dopaminergic receptors by chlorpromazine leads to increased prolactin and melatonin levels (14). Both high prolactin and melatonin levels may be protective against breast cancer (14).

#### *The possible role of melatonin in breast cancer*

The role of melatonin in the aetiology of breast cancer is controversial with both high (15, 16) and low (17) melatonin levels being suggested as promoting the development of breast cancer. Melatonin is known to inhibit ovarian oestrogen production, pituitary gonadotrophin production and sexual development and maturation. Therefore low melatonin levels may be associated with prolonged oestrogen excess in females. This hypothesis is supported by reports that female psychiatric patients taking chlorpromazine have a lower incidence of breast cancer than the normal population. One effect of chlorpromazine is to raise serum melatonin levels (17). Melatonin receptors have also been demon-

strated in the human ovary suggesting a direct influence of melatonin on ovarian function (17). High melatonin levels suppress mammary tumour growth (14). Melatonin stimulates the synthesis of a thromboxane  $A_2$  like substance. Agents that stimulate thromboxane  $A_2$  synthesis or action may cause decreased growth (14).

Plasma melatonin,  $T_3$  and thyroxine ( $T_4$ ) levels have recently been studied in women with breast cancer. Plasma melatonin and  $T_3$  levels were significantly increased.  $T_4$  levels were significantly decreased (15). The authors of the study concluded that both melatonin and thyroid hormones, in addition to oestrogen, may be important in the aetiology of breast cancer.

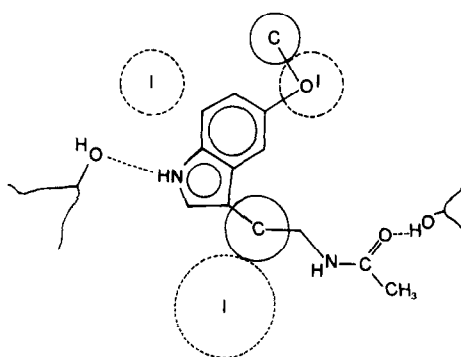


Fig 1 Proposed binding of melatonin to a  $rT_3$  based melatonin receptor

The present author has previously proposed that certain serotonergic receptors are  $T_2$  and reverse tri-iodothyronine ( $rT_3$ ) based (13, 18). As melatonin is a metabolite of serotonin it can easily be appreciated that melatonin receptors may be  $T_2$  and  $rT_3$  based as well. The proposed binding of melatonin to a  $rT_3$  based receptor is shown below. Figure 1 shows the size and position of the three iodine atoms of  $rT_3$  in the plane separating the 3', 5' di-iodophenolic ring of  $rT_3$  from the aromatic system of melatonin, as has been described previously by the present author (18). The position of the hydrogen bonding exoreceptor binding sites are also shown. It is the position of these exoreceptor binding sites that make this  $rT_3$  based receptor specific for melatonin. The 3 iodine atom on the lower alanine bearing ring of  $rT_3$  is projecting out from the page, perpendicularly to the plane of the 3', 5' di-iodophenolic ring of  $rT_3$  as discussed previously (18).

$rT_3$  levels parallel  $T_4$  levels and are low in hypothyroidism (19). This could possibly be associated with a decreased  $rT_3$  based melatonin receptor density. This would lead to decreased responsiveness to melatonin, in spite of the high circulating melatonin levels observed in breast cancer patients (15). The melatonin would therefore be unable to exert its anti-oestrogenic and anti-noeplastic effects. The situation would be analogous to decreased beta adrenergic responsiveness in hypothyroidism in spite of high circulating noradrenaline levels (20). Hypothyroidism is associated with decreased beta adrenergic receptor density (10). The present author has previously proposed that the beta<sub>1</sub> adrenergic receptor is  $T_3$  based (13).

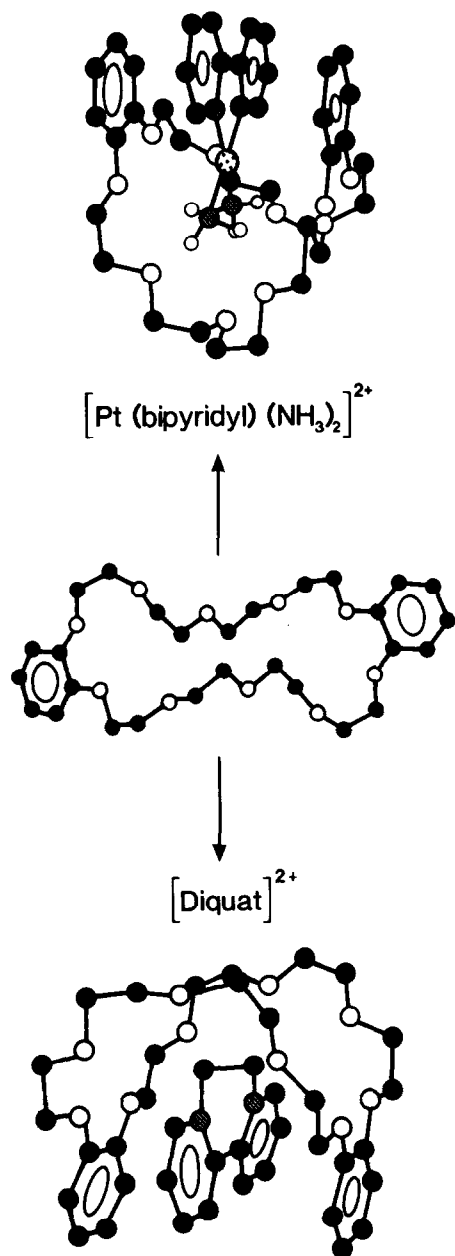
#### *Prolactin, dopamine and thyroid hormones in breast cancer*

Phenothiazines increase prolactin and melatonin levels (14). Female psychiatric patients on chlorpromazine have a lower incidence of breast cancer than the normal female population (17). This may be secondary to raised prolactin and melatonin levels (14). This theory is, however, not universally accepted as some tumours induced by dimethylbenzanthracene (DMBA) in rats show increased malignancy after both melatonin and prolactin administration (21).

The effects of altered thyroid hormone status are complex as explained earlier. Hypothyroidism is generally associated with raised prolactin levels (1), mainly due to decreased metabolism of prolactin. A study also concluded that any relationship between decreased thyroid function and breast cancer is not mediated through increased prolactin secretion (22). The importance of prolactin in the aetiology of breast cancer is therefore uncertain.

#### *$T_2$ as part of the ligand binding site of the oestrogen receptor*

Oestradiol and the catechol oestrogens, like the catecholamines, and serotonin are aromatic molecules with phenolic or catechol hydroxyl substituents. The aromatic rings of these molecules are electron rich and would be expected to complex well with an electron deficient aromatic system in the receptor as explained previously by the present author (13, 18). This led the present author to propose that iodotyrosine and iodothyronine molecules may be incorporated into the ligand binding site of the various catecholamine



**Fig 2** The binding of DB30C10 to [Pt(Bipyridyl) (NH<sub>3</sub>)<sub>2</sub>]<sup>2+</sup> and Paraquat (with permission), from the 'New Scientist', London, The Weekly Review of Science and Technology 1 May 1986

Colour code

Platinum atom

Nitrogen atoms

Oxygen atoms

Carbon atoms

— stippled

— cross hatched

— open rings

— black circles

and serotonergic receptors (13, 18). Following similar logic it can easily be appreciated that oestrogen would be expected to bind well to a 3,3' di-iodothyronine based receptor. The forces binding oestrogen to its receptor are all secondary forces, including hydrogen bonding and the charge transfer effect, whereby an electron rich molecule transfers its electron density to an electron deficient molecule, generating an electrostatic attraction (23).

Examples of charge transfer interactions between aromatic systems have been reviewed (23). The electron donor discussed is the large polycyclic ether Dibenzo 30 Crown 10 (DB30C10). The electron rich aromatic rings of DB30C10 are shown forming charge transfer complexes with the electron deficient aromatic systems of the platinum complex [Pt (Bipyridyl)(NH<sub>3</sub>)<sub>2</sub>]<sup>2+</sup> and the herbicide diquat. [Pt (bipyridyl)(NH<sub>3</sub>)<sub>2</sub>]<sup>2+</sup> is a derivative of the anticancer drug cisplatin. Both charge transfer complexes are illustrated below. The structure of the complexes is maintained in solution (23).

The cyclic crown ethers were synthesized serendipitously by Charles Pedersen. He mixed catechol and dichlorodiethylether in NaOH and produced DB18C6 (24). Therefore the electron donating aromatic systems of the dibenzyl crown ethers are derived from catechol, as are the electron donating systems of the catecholamines and catechol oestrogens.

Catechol oestrogens may be obligatory mediators of the oestrogen action in the central nervous system (25) and are metabolites of oestrogens in many tissues including the hypothalamus, pituitary and liver. 2OH oestradiol, a catechol oestrogen, binds to dopaminergic receptors (26). It therefore acts as a D<sub>2</sub> dopaminergic antagonist and this may be the mechanism whereby it increases prolactin secretion (27). This suggests structural similarities between the oestradiol receptor, catechol oestrogen receptor and the D<sub>2</sub> dopaminergic receptor. Furthermore the D<sub>2</sub> dopaminergic agonist bromocryptine and the D<sub>2</sub> dopaminergic antagonist sulpiride bind well to oestradiol receptors, having one tenth the affinity of oestradiol for the receptor (28). Oestradiol also displaces alpha<sub>1</sub> adrenergic antagonists from alpha<sub>1</sub> adrenergic receptors. The present author proposes that the common structural feature in all these receptors is that they are all 3,3' T<sub>2</sub> based.

Oestradiol also has a close structural relation-

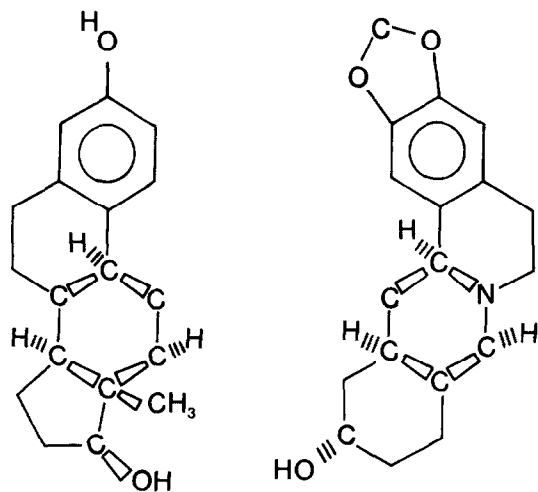


Fig 3 Close structural similarity between oestradiol and CH38083

ship to CH38083. CH38083 is a potent, selective  $\alpha_2$  adrenergic antagonist (29).

The major differences between the two molecules are the nitrogen atom in CH38083 which may be positively charged at physiological pH, and the position and orientation of the nonphenolic hydroxyl group. Both the above bear a close structural relationship to the potent polycyclic aromatic carcinogen 2 hydroxybenzo(a)pyrene (20HBP). 20HBP binds to oestrogen receptors (30). The similarity between 20HBP and oestradiol led to the hypothesis that 20HBP may bind to the oestrogen receptor and be translocated to a specific DNA locus once activated to a diol epoxide (36). 20HBP induced breast cancer might therefore result from a site specific reaction with DNA rather than from the indiscriminate attachment of the carcinogen to DNA (30).

20 Methylcholanthrene and 7, 12 DMBA also bind to the oestrogen receptor and specifically induce tumours in the mammary glands, ovary and uterus. These three sites have a very high concentration of the oestrogen receptor while other tissues do not. A high dose of oestradiol administered simultaneously with these carcinogens protect the animals from the induction of tumours. This effect has been attributed to competition between oestradiol and the carcinogens for binding to the oestradiol receptor protein (31). It has also been shown that there is an acceleration of induction of breast neoplasms by DMBA in hypothyroid or iodine

deficient rats but this effect only occurs when the iodine deficiency or hypothyroidism is induced prior to the administration of the carcinogen (32).

Oestrogen would be expected to bind well to a 3,3'  $T_2$  based oestradiol receptor just as CH38083 would be expected to bind well to a 3,3'  $T_2$  based  $\alpha_2$  adrenergic receptor. The proposed binding of oestradiol and CH38083 to their respective  $T_2$  based receptors is shown below.

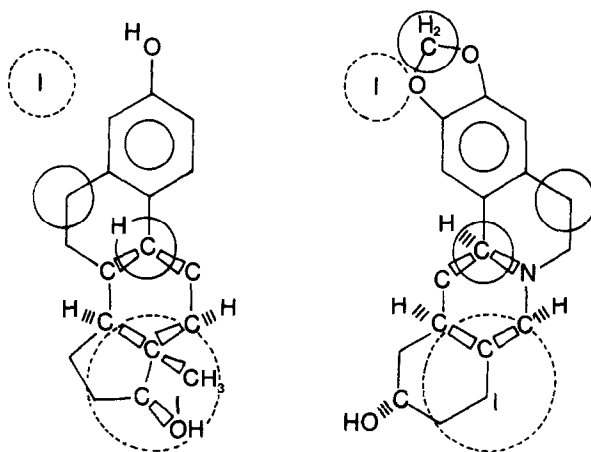


Fig 4 Proposed binding of oestradiol to a  $T_2$  based oestrogen receptor

Proposed binding of CH38083 to a  $T_2$  based  $\alpha_2$  adrenergic receptor

Note the importance of the chair conformation of the hydrogenated "C" ring of oestradiol and of the corresponding ring of CH38083. This structural feature is necessary to accommodate the bulky hydrophobic 3 iodine atom on the lower alanine bearing ring of 3,3' $T_2$ . 20HBP is a completely planar aromatic molecule and the 3 iodine atom and the alanine bearing lower aromatic ring of  $T_2$  must be displaced by rotation on the ether linkage of  $T_2$  allow the binding of 20HBP. This, together with decreased hydrogen bonding may be the reason for the low affinity of 20HBP for the oestradiol receptor.

Szent Gyorgi proposed that polycyclic aromatic hydrocarbons may exert their carcinogenic effect by being electron donors in a charge transfer interaction with an electron deficient electron acceptor (33). His theory was extensively developed by Pullman and Pullman (34). Szent Gyorgi

also proposed that thyroxine ( $T_4$ ) could act as an electron acceptor in a charge transfer interaction and postulated that many of the similarities in the effects of 2,6 dinitrophenol and  $T_4$  were due to this property (25).

$T_3$  and  $T_4$  have a rigid structure due to the presence of the 3,5 di-iodosubstituents on the lower alanine bearing ring (36). 3,3'  $T_2$  is therefore the most suitable iodothyronine to act as a receptor for oestradiol due to its flexible nature (37). The proposed binding of oestradiol to a  $T_2$  based oestrogen receptor is illustrated below.

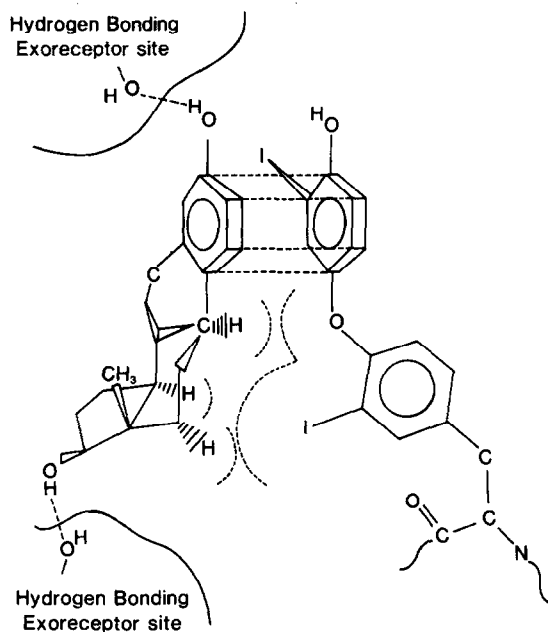


Fig 5 Proposed binding of oestradiol to a  $T_2$  based oestrogen receptor

#### *The post translational modification of tyrosine*

There are three ways in which tyrosine is post translationally modified. The phenolic hydroxyl group of tyrosine may be phosphorylated or sulfated. The other post translational modification is iodination to the iodothyronines and hence formation of the iodothyronines.

The oestrogen receptor protein contains four tyrosyl residues. One is incorporated into the hydrophobic hormone receptor domain and two are involved in the binding of the oestrogen — oestrogen receptor complex to its nuclear receptor (38).

Phosphorylation on tyrosine confers hormone binding ability to the oestradiol receptor (38).

Anti-phosphotyrosine antibodies have been prepared which bind to the oestradiol — phosphorylated oestrogen receptor complex.

The antiphosphotyrosine antibody does not seem to displace [ $H^3$ ] oestradiol from the [ $H^3$ ] oestradiol — oestradiol receptor complex, but seems to bind to one of the other phosphorylated tyrosine residues. This suggests to the present author that the tyrosyl residue in the hydrophobic domain required for hormone binding is not phosphorylated. (Phosphorylation of this tyrosine would make this site less hydrophobic). Instead the present author proposes that this tyrosyl residue is post translationally iodinated by hydrophobic iodine atoms to form monoiodotyrosine and then possibly converted to 3,3' di-iodothyronine.

#### *A possible mechanism for the synthesis of $T_2$ based oestrogen receptors*

Iodothyronines are known to be incorporated into various iodoproteins (39, 40). There are no codons in DNA for the iodothyronines and at present the mechanism for incorporation of iodothyronines into iodoproteins has not been elucidated. It may involve post translational modification of tyrosine.

The mammary gland is known to concentrate iodine against a concentration gradient (1). Breast cancer tissue has been shown to take up more  $I^{125}$  and  $I^{131}$  than normal breast tissue (41). Extra-thyroidal  $T_4$  has been found in completely thyroidectomised rats following injection of radio-iodine (42).  $T_4$  can be produced outside the thyroid by iodination of tyrosine by non specific peroxidases including lactoperoxidase (43). Lactoperoxidase is also used experimentally to trace iodinate immunoglobulins and other proteins that have tyrosyl residues (44). The peroxidases in breast tissue could therefore be required for iodination of the oestrogen receptor tyrosyl residue to produce a  $T_2$  based oestrogen receptor. Oestrogen is known to markedly and specifically increase peroxidase levels in oestrogen responsive tissues such as the breast and the uterus (45). Non oestrogen dependant peroxidase is also found in breast tissue (46). Breast carcinoma tissues are presently being examined for features of oestrogen responsiveness such as progesterone receptor activity and notably oestrogen induced peroxidase activity (47). Attempts are also being made to correlate peroxidase activity with the malignant potential of breast carcinomas (46).

Oestrogen controls oestrogen receptor synthesis (48), as well as induces peroxidase activity (45). The present author believes these two processes are necessarily linked as the oestrogen receptor protein tyrosyl residue in the ligand binding site must be iodinated to mono-iodotyrosine and then converted to 3,3' di-iodothyronine.

#### *The relationship of free $T_4$ levels to breast cancer*

It has recently been shown that there is a high inverse, linear correlation between free  $T_4$  and the risk of breast cancer (49). Thyroid function is higher in normal Japanese women (at low risk) than in British women (at high risk). There is a highly significant inverse correlation ( $r = 0.96$   $p < 0.001$ ) between median  $FT_4$  values and either the absolute incidence of breast cancer, or of relative risk where the incidence in mainland Japanese has been set at unity and the rates of the remaining groups expressed in relation to this (49).

The population of women in the study were: Mainland Japanese (MJ), Hawaiian Filipinos (HF), Hawaiian Japanese (HJ), Hawaiian Chinese (HChi), British (UK), Hawaiian (H) and Hawaiian Caucasians (HC).

The authors ask whether it is possible that small differences in thyroid function could be of importance in the aetiology of breast cancer (49). The present author's reply is affirmative as he proposes that iodination of the tyrosyl residue in the hydrophobic oestrogen binding site in the oestrogen receptor occurs. Thomas et al (49) propose an additional mechanism. The ratio of

5 alpha to 5 beta reduced androgen metabolites is a sensitive biological marker of thyroid hormone action. Japanese women have greater amounts of 5 alpha metabolites in their urine than British women. Since the 5 alpha metabolite is a potent inhibitor of aromatase activity, an enzyme important in oestrogen biosynthesis, minor alterations in thyroid functioning may have an important effect on oestrogen status when populations are considered (49). In addition hypothyroidism is associated with a raised free oestradiol level and lower SHBG levels than normal. The reverse is found in hyperthyroidism (7). Hypothyroidism or even low  $FT_4$  levels are associated with a hyperoestrogenic state. It may also be found that hypothyroidism leads to increased oestrogen receptor density as explained earlier. The effect of raised oestrogen levels in hypothyroidism may well be amplified by this mechanism.

#### *Low $FT_4$ levels in the management of breast cancer patients*

If low  $FT_4$  levels are found to be associated with increased oestrogen receptor density, then lowering  $FT_4$  may place certain breast tumours in more favourable prognostic groups by increasing oestrogen receptor density and hence possibly progesterone receptor density as well. There is a well established inverse relationship between steroid receptors and tumour growth rates (53). This would be expected to be most effective with patients who have no endogenous oestrogen production and are being treated with tamoxifen.

Other benefits can be expected from inducing low  $FT_4$  levels including increased  $D_2$  receptor density (11) which will potentiate the antiemetic of  $D_2$  dopaminergic antagonists such as sulpiride. Sulpiride also has a high affinity for oestradiol receptors (28) and would therefore complement tamoxifen therapy to some extent. Hypothyroidism also potentiates the effects of narcotic analgesics (50) needed in the management of breast cancer.

A possible drawback of hypothyroidism is the expected increase in depression associated with hypothyroidism (51). This should be controlled with a tricyclic anti-depressant.

Many women with breast cancer develop low  $FT_4$  levels (15) and this fact should be exploited therapeutically. However this may be accompanied by high  $T_3$  levels (15). This would have the effect of increasing beta adrenergic responsiveness in

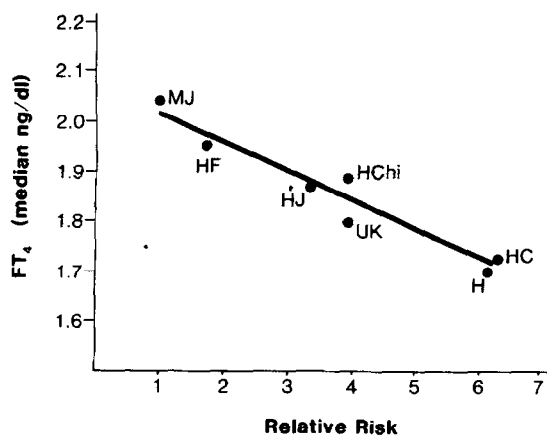


Fig 6 Relationship between  $FT_4$  and relative risk of breast cancer (with permission)

favour of  $\alpha_1$  adrenergic,  $D_2$  dopaminergic, opiate and serotonergic responsiveness. Better therapeutic responsiveness should be obtained when  $T_3$  levels are kept low.  $rT_3$  supplementation may be beneficial. By keeping  $rT_3$  levels high, the density of certain melatonin receptors may be increased, allowing the high melatonin levels to exert an antineoplastic effect. It has been noted in some patients with non thyroidal illnesses, including cancer, a syndrome develops with low  $T_4$  and low  $T_3$  levels. Concentration of  $rT_3$  is usually high due to reduced metabolic clearance. These changes in thyroid hormone levels in non thyroidal illness may have a homeostatic significance (19). The present author proposes that this homeostatic adjustment will have significant effect on various monoamine, oestrogen and progesterone receptor densities which can be exploited therapeutically, leading to more effective adjuvant therapy for oestrogen dependant tumours and hopefully to better survival figures.

## Conclusion

Subtle defects in thyroid function are a feature of breast cancer and do exert a minor but not negligible effect on the incidence of breast cancer and its clinical course (1). Large studies may be required to detect with certainty such an effect (1). There are enough pointers in the literature to suggest that such an effort would be worthwhile for its contribution to our knowledge of the natural history of breast cancer and for its potential in future attempts to reduce incidence and prolong disease free survival of breast cancer (1).

Increased iodine intake or increase in thyroid function may eventually reduce the incidence of breast cancer (6). So far iodine supplementation in iodine deficient areas of the U.S.A. has led to a sharp decline in the incidence of goitres, while the breast cancer incidence has so far remained unchanged (52). A possible explanation for this paradox is that breast structural changes due to iodine deficiency, may have occurred in young women before supplementation was used. A fall in later generations might ultimately be expected (1).

The oestrogen receptor has been purified to near homogeneity. The basic 4S steroid binding site has molecular weight of 65 000 daltons and contains one oestradiol binding site (48). The present author is currently involved in research

to determine the iodine content of the purified oestrogen receptor protein as well as investigating the possible regulation of oestrogen and progesterone receptor density by hypothyroidism and hyperthyroidism. This work may have important implications for understanding the physiology of the oestrogen receptor and the role of iodine and thyroid hormones in the aetiology of cancer of the breast, uterus and ovary.

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